DISSERTATION

INTRASPECIFIC VARIATION IN REPRODUCTIVE ECOLOGY AND SUCCESS OF A

KEYSTONE STREAM FISH, BLUEHEAD CHUB

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2019

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ABSTRACT

INTRASPECIFIC VARIATION IN REPRODUCTIVE ECOLOGY AND SUCCESS OF A

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Individuals differ in many traits, and such variation affects reproductive tactics to

maximize fitness. Variation in reproductive success influences not only population dynamics and

genetic structure, but also community and ecosystem structure when the species plays an

important functional role such as keystone species. Key topics in investigations of individual

variation are to quantify the degree of variation and its implications on fitness, which are the

ultimate goals in my research focusing on reproductive ecology of a keystone stream fish,

bluehead chub *Nocomis leptocephalus*.

Understanding the timing of spawning is a key topic in fish ecology because reproduction

is a determinant of life history and population structures. The timing of spawning has evolved to

maximize offspring survival in response to optimal biotic and abiotic conditions. However,

environmental control on spawning timing, particularly in fine spatial scale (e.g., local scales)

and temporal scales (e.g., daily trends), are poorly understood for most native stream fishes.

(Chapter 1) The first chapter aimed to characterize spawning periodicity of bluehead chub and

yellowfin shiner among local streams. I conducted daily observations to address daily spawning

patterns and examine the impacts of environmental drivers such as water temperature and flow

conditions. I expected that the timing of spawning would be synchronous among local streams if

regional factors, such as weather patterns, are the key drivers of reproduction. Results showed

that fish spawned periodically within the same stream and across different streams. Water

temperature affected the timing of spawning in all streams, whereas the effects of flow varied by stream. Specifically, spawning was triggered by a short-term increase in water temperature and observed under stable flow conditions.

While environmental fluctuations affect daily spawning patterns, these may play an important role in variation in nesting behavior and reproductive interactions with nest associates. Nest construction is a distinct reproductive strategy, which can attract females and maximize offspring survival, and this can be affected by environmental fluctuation and resource availability. Further, understanding how nesting behavior attracts nest associates is crucial for understanding how nesting behavior may determine positive reproductive interactions (i.e., nest association). However, questions still remain about how abiotic and biotic factors affect variation in nest size and interactions with yellowfin shier. **(Chapter 2)** I investigated the effects of abiotic and biotic factors on nest size and utilization by associates, which were related to the reproductive success of bluehead chub. I developed a conceptual hypothesis of causal relationships among abiotic and biotic factors and two key variables (i.e., nest size and yellowfin shiner abundance), then which I tested multiple hypotheses using a path analysis. The results showed that male size was positively related to various biotic variables, including nest sharing, duration of spawning, and re-use of nesting resources. Larger male size and nest sharing behavior led to larger nest size and attracted more yellowfin shiner.

While various abiotic and biotic factors affected nesting behaviors and reproductive interactions, these findings (Chapter 2) considered the fitness proxies but not individual fitness such as reproductive success (i.e., the number of offspring sired by each individual). Although individual traits play the main role in reproductive success, studying reproduction in the wild is notoriously difficult due to the cryptic spawning and aggregation of multiple individuals.

(Chapter 3) For my third chapter, I investigated how individual variation led to differences in mating system and reproductive success in a natural stream using passive integrated transponder (PIT) tags and microsatellite markers. I hypothesized that larger males would reproduce more successfully than smaller males in a field experiment, but other individual traits such as cooperative behavior could mediate the size-dependent reproductive success in the natural stream. I predicted that female reproductive success is associated with body size, but the female choice could mediate this relationship. Lastly, I expected some key individual traits such as body size and cooperative nest sharing to lead to successful nest construction, which could influence reproductive success. I observed that nest size and nesting effort led to successful reproduction in males, and nest sharing (i.e., the number of males on nests) was an important behavioral trait that led to successful nest construction and attracting females. However, I found little evidence that male body size had significant effects on reproductive success and nest construction, while body size was positively associated with female reproductive success. Overall, my findings suggest that male body size is not always a primary factor for constructing larger nests, female choice, or reproductive success. Perhaps cooperative behavior (i.e., nest sharing) could mediate the size-dependent reproductive success.

Collectively, my three chapters advance reproductive ecology of a keystone stream fish, bluehead chub. My dissertation research demonstrates that two key environmental variables (i.e., water temperature and flow) associated with weather conditions have a major influence on spawning activities and nesting behaviors lead to variation in reproductive outcomes. Chapter 1 demonstrated that fish responded to a range and short-term increase of environmental cues. Chapter 2 and 3 demonstrated the relative importance of environments and individual traits (i.e., body size and nesting behavior) to nest construction, female choice, and reproductive outcomes.

While male body size was a significant factor for constructing larger nests in Chapter 2, I found little evidence that body size was determinant of reproductive success in Chapter 3. Environmental impacts could mediate the size-dependent reproductive success, but the notable pattern was that cooperative reproduction among males (i.e., nest sharing) was the most important factor leading to successful reproduction. This is one of the few studies that demonstrates male-male cooperation in nest-building fishes, because occupying and defending nests through aggressive behaviors are common reproductive strategies in nest-building males. Further, my findings have valuable insights in complex relationships among males; they act cooperatively but competitively engage in efforts to increase fitness that may cause reproductive skew within a cooperative male group.

Individual variation provides the very basis for evolutionary adaptation and population resiliency, which are critical in species conservation as organisms respond to environmental changes. However, basic biology and life history are still poorly understood on native freshwater fishes, while this knowledge is critical for conservation and management of stream ecosystems. This study illustrates that reproductive ecology and success were affected by a complex interplay of environmental conditions, phenotypic variation, and behavior in bluehead chub, whose nests are also used by other fish species in the Southeast USA. In addition, my study designs, including intense observations and monitoring nests using PIT antenna array coupled with genetic analysis, would shed light on a novel approach in behavioral ecology and fisheries research.

ACKNOWLEDGEMENTS

Lastly, I thank bluehead chub, yellowfin shiner, and beautiful environments in South

Carolina. I owe them all so much, and I will never forget what I have learned from them.

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CHAPTER 1: SPAWNING PERIODICITY AND SYNCHRONY OF BLUEHEAD CHUB

AND A NEST ASSOCIATE, YELLOWFIN SHINER, ACROSS LOCAL STREAMS

1. Introduction

Fish spawning is strongly regulated by external conditions. Timing, duration, and

frequency of reproduction have evolved to maximize offspring survival in response to

availability of prey abundance, competition among juveniles, and long-term environmental

conditions (Mills 1991; Bestgen et al. 2006; Divino and Tonn 2007; Morisette et al. 2009).

Spawning is often triggered by broad-scale environmental factors such as climate, which leads to

spatial synchrony among local sites in a region (Cattanèo et al. 2003; Engen and Saether 2006;

Chevalier et al. 2014). However, environmental control on spawning timing, particularly on fine-

scale temporal scales (e.g., daily or over several days), is poorly understood for most non-game

stream fishes.

Spawning of stream fishes is a complex process, triggered by a suite of environmental

variables operating at different temporal scales (Morisette et al. 2009; Forsythe et al. 2012; King

et al. 2016). Stream fish initiate and discontinue spawning under a specific range of temperatures

(Bruch and Binkowski 2002; Reid 2006; King et al. 2016), flow conditions (Falke et al. 2010b;

Forsythe et al. 2011; Peoples et al 2014), and photoperiod (Falke et al. 2010a; Forsythe et al.

2012). Some of the environmental variables change predictably within a spawning season (e.g.,

photoperiod), but others such as temperature and flow change more stochastically from day to

day (Forsythe et al. 2012; Straight et al. 2015). The short-term variation, including the rate of

change in temperatures and flow, trigger spawning of stream fishes within the spawning season

(Paragamian and Wakkinen 2002; Forsythe et al. 2011). This fine-scale environmental variation

can lead to spawning periodicity in which spawning occurs on some days at intervals, but not on others, within a broadly defined spawning season (Forsythe et al. 2012).

Fine-scale temporal cues of spawning have been much less studied compared to broad-scale environmental triggers in stream fishes, but this knowledge is important to planning aquatic conservation. Studies of the timing of spawning have focused on larval emergence, reproductive migration, and their inter-annual and among-site variation (Reid 2006; Falke et al. 2010a; Krabbenhoft et al. 2014; Catalano and Bozek 2015; Straight et al. 2015; King et al. 2016). Direct observations of spawning are typically challenging in fishes, and daily spawning patterns are inferred based on larval counts, the number of nests (e.g., salmonid redds), and detection of migratory adults (Paragamian and Wakkinen 2002; Divino and Tonn 2007; Falke et al. 2010a ; Forsythe et al. 2012; Warren et al. 2012). Flow and thermal regimes in river ecosystems play vital roles in the reproductive timing and outcomes of stream fishes (Poff et al. 1997; Steel et al. 2015). Human activities (e.g., urbanization and hydropeaking dams) affect components of flow and thermal regimes on time scales of days, and lead to abnormal spawning patterns and reproductive failures (Santucci and Wahl 2003; Warren et al. 2012; Steel et al. 2015; Kemp 2017). Filling the knowledge gap of fine-scale spawning pattern is important for understanding the effects of environmental cues on the reproductive ecology of stream fishes and managing for aquatic conservation.

Bluehead chub (*Nocomis leptocephalus*) provides a unique system to study environmental influences on periodicity within a spawning season because of their prolonged spawning season (2 – 3 months) and conspicuous nests that make direct observations of spawning possible. Bluehead chub are widely distributed throughout the Atlantic slope of North America (Lanchner 1952). Male bluehead chub move pebbles to construct dome-shaped nests as

a host species (Wallin 1989; Sabaj et al. 2000), and nest associates spawn on the nests with them (Jenkins and Burkhead 1994; Jonhston 1994a). This interaction (i.e., nest association) is mutually beneficial because bluehead chub extend parental care to nest associates, which in turn provide a dilution effect when predators invade bluehead chub nests (Vives 1990; Johnston 1994b; Peoples et al. 2016). In the upper Piedmont region of the southeastern USA, bluehead chub interact reproductively with yellowfin shiner (*Notropis lutipinnis*) via nest association (Wallin 1989; 1992; Marcy et al. 2005). Both fishes spawn from spring to early summer at water temperatures 16 – 23°C (Wallin 1989; 1992). Wallin (1989) observed that spawning did not occur continuously, but exhibited a periodic pattern with clusters of days of spawning followed by consecutive days without spawning. However, little is known about how environmental cues affect spawning periodicity of bluehead chub and yellowfin shiner. Understanding spawning periodicity of a keystone species (i.e., bluehead chub) would provide valuable insights into community structure and dynamics because nest associates rely on keystone species for reproduction (Wallin 1992; Peoples et al 2016; Peoples and Frimpong 2016).

In this study, I conducted field observations for 89 consecutive days to characterize daily spawning patterns of bluehead chub and yellowfin shiner at three streams in the Piedmont region of South Carolina, USA. The study species enabled us to understand their daily spawning periodicity because field observations were facilitated by conspicuous pebble mound nests constructed by bluehead chub, which attracted swarms of brilliantly-colored breeding nest associates (Wallin 1989; Sabaj et al. 2000; Peoples at al. 2015). The objectives of this study were to (1) document reproductive periodicity of both species, (2) examine reproductive synchrony among local streams, and (3) assess whether daily variation in spawning is explained by environmental variables. I expected that spawning would take place synchronously among local

streams if regional factors, such as weather patterns (i.e., temperature and precipitation), are the key drivers of bluehead chub and yellowfin shiner reproduction.

2. Methods

2.1 Study area

This study was conducted in three streams in the Clemson University Experimental Forest located in the upper Piedmont region of South Carolina, USA (Fig. 1-1). Study streams were selected to represent a gradient of stream size and habitat characteristics in which both species occurred. Indian Creek was the smallest stream based on mean wetted width (2.7m) (Table 1-1). The stream canopy was mixed hardwoods and dominant substrates were pebble (16 – 64 mm) and sand (0.01 – 2 mm) (Table 1-1). Todd Creek was intermediate in size (wetted width = 3.8 m) with an open canopy (Table 1-1 and Fig. 1-1). Substrate composed of a combination of bedrock, cobble, pebble, and sand. Sixmile Creek was the largest of the three streams (wetted width = 7.3 m), and was mainly covered by overhanging trees but some parts of the stream had little shade from riparian shrubs (Table 1-1). The dominant substrate was sand and gravel (2 – 16 mm). The length of study area was 1.6 km in Indian Creek, 1.0 km in Sixmile Creek, and 0.8 km in Todd Creek. Common species found in all streams were bluegill (*Lepomis macrochirus*), creek chub (*Semotilus atromaculatus*), green sunfish (*L. cyanellus*), redbreast sunfish (*L. auritus*), and striped jumprock (*Moxostoma rupiscartes*). Rosyface chub (*Hybopsis rubrifrons*), another known nest associate (Marcy et al. 2005), was found in Indian and Sixmile Creeks, but they were much less common than yellowfin shiner.

2.2 Field sampling

Spawning activity of bluehead chub and yellowfin shiner was observed for 89 consecutive days from April 4th to July 1st, 2016. The study period encompassed an entire spawning season because the field observation had started nearly two weeks before the first nests were observed and it ended one week after the last nests had been observed (see Results). Observations were conducted between 10 am and 5 pm. To avoid a bias introduced by observation hour, the order of visits to streams were randomized daily. Based on observations of the first five nests every 4 hours for 24-48 hours, I determined that nests stayed active for more than a day (S. *Kim unpublished data*), and the sampling protocol was unlikely to miss active nests. Two observers walked the bank or in the stream channel to locate bluehead chub nests. Male bluehead chub prefer shallower runs covered by gravel and pebble for nesting (Wallin 1989; Sabaj et al. 2000; Bolton et al. 2015), but observers checked all other habitat types including runs, riffles, pools and undercut banks. Observers confidently identified bluehead chub nests because the pebbles mounds were conspicuous and easily viewed in the clear water. When a new nest was located, a marker was placed on the nearest bank with a unique identification number. Observers checked marked nests daily until they were destroyed by high flows or male bluehead chub stopped attending nests. Yellowfin shiner display brilliant breeding colors (i.e., red body color with yellow fins) while spawning on bluehead chub nests (Marcy et al. 2005), and yellowfin shiner spawn only when bluehead chub spawn or attend their nests (Wallin 1989; 1992). Thus, I recorded an active nest when yellowfin shiner with breeding color were schooling or spawning on the nest. The number of active nests was recorded daily at each study stream.

Regional- and stream-scale environmental data were collected daily including precipitation (mm) and air temperature (°C) measurements from the nearest weather station (Clemson-Oconee County Airport Weather Station by the National Weather Service),

approximately 10 km from the study area. Water temperature (°C) and level (m) were recorded

hourly by deploying loggers (Model U20L-004, HOBO Onset Computer Corp., Bourne,

Massachusetts) at the downstream end of each stream. Stream flows were not gaged in any of my

study streams, and I considered that water level represented temporal variation in stream flows.

2.3. Statistical analyses

2.3.1 Spawning synchrony among streams

The degree of reproductive synchrony among three streams was analyzed using the non-

parametric Spearman's rank correlation test with a Bonferroni correction of a statistical

significance level (adjusted $\alpha < 0.017$, or 0.05 divided by three pairwise comparisons). The test

was based on the cumulative number of daily active nests between each pair of streams.

Statistical significance was set at $\alpha < 0.05$ for all analyses that follow.

2.3.2 Environmental effects on spawning

The link between daily spawning and environmental variables was examined by using

time-series models. A generalized linear auto-regressive moving average (GLARMA) model

(Benjamin et al. 2003) was applied to assess the effect of environmental variables on spawning

periodicity. The GLARMA model includes auto-regressive moving average (ARMA)

components to account for the serial dependence in time series data using regressors that are

conditional on past values (Dunsmuir and Scott 2015). Unlike the conditional ARMA model, the

GLARMA model is a non-Gaussian state space model which can apply link functions (e.g.,

Poisson distribution), analogous to the generalized linear model (GLM), by using time-series

explanatory variables to account for temporal variation in a response variable (Benjamin et al.

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2003; Dunsmuir and Scott 2015). For instance, let Y_t represent a serial observation over time t (t = 1, … , 89: the number of daily observations in this study) and an array ($x_{t,k}$) containing regressor k (i.e., explanatory variables) at time t. Let $F_t = (Y_{1:t-1}; x_{1:t,k})$ be the past information on the response series and the past and present information on the regressors. For the Poisson GLARMA, the conditional distribution of Y_t given F_t is assumed to follow a Poisson distribution with mean μ_t:

$$Y_t|F_t \sim \text{Poisson } (\mu_t) \qquad\qquad \text{equation (1)}$$

$$\log(\mu_t) = \alpha + \boldsymbol{\beta}_k x_{t,k} + Z_t \qquad\qquad \text{equation (2)}$$

where α is an intercept, $\boldsymbol{\beta}_k$ is a vector of regression coefficient of length k, and Z_t accounts for a serial dependence in the response process. The binomial GLARMA model assumes that the distribution of the Y_t given $x_{t,k}$ and F_t is denoted by:

$$P(Y_t = 1|F_t) = p_t \qquad\qquad \text{equation (3)}$$

$$\text{logit}(p_t) = \alpha + \boldsymbol{\beta}_k x_{t,k} + Z_t \qquad\qquad \text{equation (4)}$$

where $Y_t = 1$ denotes the presence of active nests and p_t is the probability of presence during time t. The GLARMA model includes auto-regressive (AR) and moving average (MA) components by specifying Z_t as follow:

$$Z_t = \sum_{j=1}^{\infty} \gamma_j e_{t-j} = \sum_{j=1}^{r} \emptyset_j (Z_{t-j} + e_{t-j}) + \sum_{j=1}^{q} \theta_j e_{t-j} \qquad \text{equation (5)}$$

where e_{t-j} is the predictive residual, γ_j is given as coefficients on ARMA term, and r and q are the orders of polynomial AR ($\emptyset$) and MA (θ) terms, respectively.

Prior to the GLARMA analysis, I conducted three steps of data examination and preparation. First, I restricted the analysis period to range from 7 days before the first nest was observed and until 7 days after the last nest was observed to focus on spawning periodicity and remove unintended influences that observations outside the spawning period may have on this

time-series analysis. Second, I only selected in-stream variables such as water temperature and level as explanatory variables water temperature was highly correlated with air temperature, and precipitation was weakly correlated with precipitation (see Results). The variance inflation factor (VIF) was used to assess multicollinearity between water temperature and level. Pairwise VIF values in three streams were lower than 3 thus both explanatory variables were retained in all streams (O'Brien 2007). Third, the GLM was fit to obtain initial values for the regression coefficient of explanatory variables and AR and MA terms, which were later used in the GLARMA analysis (Dunsmuir and Scott 2015). Response variables were the daily number of active nests in Indian and Todd Creeks, thus the Poisson GLM was applied to both streams. The binary (presence/absence) response was used in the Binomial GLM for Sixmile Creek because the daily maximum number of active nests was two (single active nest for 17 days and 2 active nests for 9 days: Fig. 1-2), and the Poisson GLARMA model did not converge. Fish typically have optimum breeding conditions bound by the lower and upper environmental thresholds (Falke et al. 2010b; Krabbenhoft et al. 2014; King et al. 2016), thus quadratic terms (in addition to linear terms) were included as predictors to account for a potentially unimodal response of spawning to environmental variables, including water temperature and water level.

The GLARMA model was used in each stream independently. Similar to the GLM, the Poisson GLARMA models were used for Indian and Todd Creek, and the Binomial GLARMA model was used for Sixmile Creek. The GLARMA models were fit using the Fisher Scoring iterative method and Pearson residuals were used to assess residual temporal auto-correlation. The initial AR and MA terms were chosen by the auto-correlation function (ACF) and partial auto-correlation function (PACF) plots using the residuals from the results of GLMs in each stream (Appendix A1). Lower orders of AR and MA terms were chosen based on the patterns

and significant residuals in the ACF and PACF plots, and polynomial terms were selected to improve model fit (Dunsmuir and Scott 2015). Candidate polynomial AR and MA terms were applied into GLARMA models, and were compared using Akaike's information criterion corrected for small sample size (AIC_c) and Akaike weights were used to identify the top-ranked and competing models (i.e., $\Delta AIC_c \leq 2$: Burham and Anderson 2002: Appendix A2). For the model diagnostics, the Wald test was applied to test whether the serial dependent parameters (ψ) were equal to zero (H_0: $\psi = 0$ versus H_a: $\psi \neq 0$), and the likelihood-ratio test was used to compare likelihoods between the GLARMA and GLM (Appendix A2). In addition, the ACF of Pearson residual plot was examined to evaluate residual temporal auto-correlation in GLARMA models (Appendix A3). Similar to the Poisson GLM, residual deviances of the Poisson GLARMA model were checked against the residual degree of freedom to assess evidence of overdispersion (Dunsmuir and Scott 2015). Overdispersion was declared if residual deviance of the GLARMA model exceeded residual degree of freedom (Hilbe 2007).

To facilitate interpretation of covariate effects in GLARMA models, explanatory variables were visually examined to discern whether spawning was correlated with their raw values or the rate of change over time. The rate of change was calculated as the difference between the present day versus the previous 1–3 days. Since the inferences varied only subtly among the intervals chosen, I reported results from the 2-day interval. The scatter plot was used to visualize the relationships between the daily number of active nests and raw value and rate of change of water temperature and level. Additionally, *t*-tests were used to assess if water temperature and level and their rates of change differed between days with or without active nests in each stream.

All statistical analyses were conducted in the R version 3.5.1 (R Development Core Team 2018), and GLARMA models were fit using the *glarma* package (Dunsmuir and Scott 2015).

3. Results

3.1 Environmental conditions

Water temperature increased gradually over the study period and highly correlated with air temperature in all streams (Spearman ρ = 0.90 – 0.92, *P*-value < 0.001: Fig. 1-2). Water temperature differed significantly among streams (one-way ANOVA: $F_{2, 183}$ = 6.5, *P*-value = 0.002). Specifically, Indian Creek (mean = 17.7 °C, range = 11.4 – 22.5) was colder than Sixmile Creek (mean = 19.1 °C, range = 11.5 – 24.8) and Todd Creek (mean = 19.2 °C, range = 12.0 – 25.0) (Tukey's honestly significant difference post-hoc test: *P*-value = 0.003 and 0.011, respectively), but there was no significant difference between Sixmile and Todd Creeks (Tukey's honestly significant difference post-hoc test: *P*-value = 0.846). Precipitation was recorded on 18 days, and the mean daily value across these days was 6.01 mm. Water level was generally declined during the study period (Fig. 1-2). There was a weak correlation between precipitation and water level (Spearman ρ = 0.06 – 0.17, *P*-value = 0.13 – 0.55), but higher water levels coincided with rain events (Fig. 2). In addition, water level in Indian Creek (mean= 0.18 m, CV= 5.41) was more temporally stable than other streams (Sixmile Creek, mean= 0.29 m, CV= 9.31; Todd Creek, mean= 0.28 m, CV= 7.61).

3.2 Spawning timing and periodicity

A total of 71 active nests (Indian Creek: 21, Sixmile Creek: 16, Todd Creek: 34) were located during the study period (Fig. 1-2). The first active nests were observed on April 17th in Sixmile Creek (14.7 °C) and on April 25th in Indian (15.8 °C) and Todd Creek (17.1 °C). Spawning was last observed on June 19th in Todd Creek and June 24th in Indian and Sixmile Creeks (Fig. 1-2). More than fifty percent of active nests were observed between April 25th and May 20th (Indian Creek, 51 %; Sixmile Creek, 60%; Todd Creek, 65%), indicating that the peak spawning time was May (Fig. 1-2 and 1-3). Active nests were recorded when water temperature ranged between 14.7 – 24.8 °C, but the range of temperature during the peak time (April 25th – May 20th) was narrower and varied by stream (Indian Creek, mean = 18.8 °C, range= 15.8 – 18.1; Sixmile Creek, mean = 19.2 °C, range= 17.6 – 20.5; Todd Creek, mean = 18.8 °C, range= 17.1 – 20.4: Fig. 1-2). Each nest was active for a median of 2 days (range= 1 – 4). Multiple active nests were typically documented on a single day in Todd Creek (median = 3 nests, range = 1 – 8), but a single active nest was most frequently located in Indian (median = 1 nest, range = 1 – 5) and Sixmile Creek (median = 1 nest, range = 1 – 2) (Fig. 1-2). Spawning was observed periodically (Fig. 1-2) at median intervals of 4 days in Indian Creek (range = 1 – 10) and Sixmile Creek (range = 3 – 7) and 6 days in Todd Creek (range = 3 – 7).

3.3 Spawning synchrony among streams

The total cumulative days that nests were active were 49 in Indian Creek, 35 in Sixmile Creek, and 65 in Todd Creek (Fig. 1-3). The cumulative percentage of daily active nests indicated that the periodicity of spawning between all stream pairs was strongly and positively synchronized (Spearman $\rho = 0.99$, *P*-value < 0.001: Fig. 1-3). Trends of water temperature and level associated with precipitation events were likely to affect local synchrony. In particular,

spawning activity was hampered by increasing flows and declining temperatures caused by precipitation (May 1st in Indian and Todd Creeks, May 12th in Sixmile and Todd Creek, May 21st in all streams, and June 4th in Sixmile and Todd Creek: Fig. 1-2).

3.4 Environmental effects on spawning

The top-ranked GLARMA models explained the periodic pattern of spawning better than competing models and GLMs (Fig. 1-4 and Appendix A2). Residual autocorrelation was not observed in all top-ranked GLARMA models (Appendix A3). Additionally, the Poisson GLARMA models had no sign of overdispersion (Indian Creek, 75.4 on 76 degree of freedom; Todd Creek, 66.4 on 73 degree of freedom), thus I focus on the description of the top-ranked models in each stream (Table 1-2). The results of GLARMA models showed that fish spawned more frequently with increasing water temperature (i.e., positive linear effects) in all streams (Indian Creek, coefficient = 11.734, P-value < 0.001; Todd Creek, coefficient= 8.887, P-value < 0.001; Sixmile Creek, 13.897, P-value = 0.019: Table 1-2), but up to a point (i.e., unimodal effects) with quadratic terms significant in Indian (coefficient = -15.340, P-value = 0.009) and Todd Creek (coefficient = -12.597, P-value < 0.001). Negative quadratic effects of water level were significant in Sixmile (coefficient= -10.206, P-value = 0.027) and Todd Creek (coefficient= -15.818, P-value = 0.021), indicating that fish spawned at the intermediate range of stream discharge but not in Indian Creek (Table 1-2).

Scatter plots showed that the number of active nests peaked at the intermediate range of water temperature in Indian and Todd Creeks, as supported by GLARMA models (Table 1-2 and Fig. 1-5). Fish spawned at the higher water temperature in Indian Creek (t-test t = -2.308, df = 74, P-value= 0.024: Fig. 1-5), but not in Sixmile (t-test t = -0.086, df = 65, P-value= 0.932) and

Todd Creek (*t*-test *t* = -0.682, df = 52, *P*-value= 0.498). Notably, the majority of spawning

occurred when water temperature had risen over the previous 2 days in all streams (Indian Creek,

t-test *t* = -2.331, df= 54, *P*-value = 0.024; Sixmile Creek, *t*-test *t* = -5.063, df = 70, *P*-value<

0.001; Todd Creek, *t*-test *t* = -3.149, df = 33, *P*-value = 0.004), as indicated by the range of

positive rate of change not overlapping or just barely overlapping with zero on days when

spawning was observed (Fig. 1-5). Water level had a significant effect on the number of active

nests in GLARMA models (Table 1-2), but there was no significant difference between days

with and without active nests (Indian Creek, *t*-test *t* = -0.850, df = 51, *P*-value = 0.399; Sixmile

Creek, *t*-test *t* = -0.008, df = 73, *P*-value = 0.994; Todd Creek, *t*-test *t* = 0.520, df = 51, *P*-value =

0.606: Fig. 1-6). Similarly, the rate of change of water level was not significantly different across

all streams (Indian Creek, *t*-test *t* = 0.048, df = 52, *P*-value = 0.633; Sixmile Creek, *t*-test *t* =

1.622, df = 79, *P*-value = 0.108; Todd Creek, *t*-test *t* = 1.123, df = 56, *P*-value = 0.225: Fig. 1-6).

However, a notable pattern was that spawning concentrated when there was little change in water

level in Sixmile and Todd Creeks, as indicated by narrower ranges of water level changes

centered around zero on days with active nests (Fig. 1-6). This result indicated that flow stability

was an important criterion for bluehead chub to spawn and flow fluctuation caused by

precipitation hampered spawning (Fig. 1-2).

4. Discussion

Bluehead chub and yellowfin shiner displayed a periodic spawning pattern that was

synchronized among local streams. Water temperature affected the timing of spawning in all

streams, whereas effects of water level varied by stream. Intriguingly, spawning concentrated

when temperature had increased over the previous two days and flow changed little (i.e., under

stable flow conditions). These findings elucidate how multiple environmental drivers determine daily spawning periodicity of stream fishes and concur with previous studies that document temperature and flow as key factors in the timing of spawning (Reid 2006; Forsythe et al. 2012; Krabbenhoft et al. 2014; King et al. 2016) and reproductive synchrony triggered by regional drivers such as weather patterns (Engen and Saether 2006; Warren et al. 2012; Chevalier et al. 2014).

My findings suggest that water temperature is likely a primary environmental cue for spawning. A fish species may spawn across a range of stream temperatures (Falke et al. 2010a; Krabbenhoft et al. 2014). Likewise, bluehead chub and yellowfin shiner spawned when water temperature ranged between 14.7 – 24.8 °C in this study, and a similar range (16 – 23 °C) was reported in a study conducted in the same major basin (i.e., Savannah River Basin) (Wallin 1989; 1992). Water temperature in Indian Creek was colder than other streams (Fig. 1-2). However, the mean and range of water temperature when fish spawned were similar to other streams because stream temperature on days with active nests was significantly higher than that on days without active nests in Indian Creek but not in others (Fig. 1-5), supporting the idea that spawning is bound by the lower and upper thermal thresholds. Additionally, the peak spawning occurred in the narrower range of temperature (15.8 – 20.4 °C), and it was nearly identical to a study of bluehead chub (15 – 21 °C) conducted in various river basins located in Georgia, North Carolina, and Virginia (Sabaj et al. 2000). Consistent ranges of water temperatures among studies suggested that stream temperature provided a thermal template that defined their spawning period. However, this alone could not address the observed periodic patterns within the spawning season. The timing of spawning of fishes is also affected by a short-term increase in water temperature (Paragamian and Wakkinen 2002; Forsythe et al. 2011; 2012). For instance,

reproductive activities of lake sturgeon (*Acipenser fulvescens*) and white sturgeon (A. *transmontanus*) are strongly associated with a temporal change in water temperature; the onset of spawning is associated with the rate of increase in temperature but a rapid decline causes fish to leave nesting sites (Paragamian and Wakkinen 2002; Forsythe et al. 2012). Similarly, the majority of spawning occurred when water temperature had risen by 2.0 °C over the previous 2 days, while there was little evidence that temperatures themselves differed between days with or without active nests in Sixmile and Todd Creeks (Fig. 1-5).

Whereas short-term warming in stream temperature triggered spawning, flow stability increased spawning activities of bluehead chub and yellowfin shiner. Flow plays a vital role in providing optimal habitat conditions for spawning (Peoples et al. 2014; King et al. 2016). Bluehead chub prefer to spawn in distinct mesohabitats (e.g., pool tails and shallower runs covered by gravel and pebble; Jenkins and Burkhead 1994; Peoples et al. 2011) and microhabtiats based on depth and velocity (Bolton et al. 2015; Peoples et al. 2016). Specifically, the range of water velocity is important for depositing eggs (Maurakis et al. 1992) and shallower depths enable bluehead chub to avoid large-bodied predators (Bolton et al. 2015). Male bluehead chub move thousands of pebbles to construct nests as a form of parental care (Wallin 1989; 1992); thus the availability of nesting resources coupled with microhabitats is known as a limiting factor for spawning activities (Peoples et al. 2014; Bolton et al. 2015; Kemp 2017). Stable flows may make it easier for male bluehead chub to locate and maintain suitable nesting sites, whereas higher fluctuations following precipitation events could hamper spawning activities. This conclusion was further supported by the finding that water level was not significant in the most hydrologically stable creek (Indian Creek: Table 1-2), but was significant in the other two creeks that experienced higher variation in flow (Fig. 1-6).

Environmental influences on spawning led to reproductive synchrony among local streams (Fig. 1-3), driven primarily by short-term increase in water temperature. Daily trends of water temperature and level were linked to weather conditions (i.e., air temperature, precipitation), which was most likely responsible for local synchrony. My data also point to the potential synchrony in spawning within the same stream, as inferred from temporarily clustered spawning patterns. This conclusion should be only incomplete because individuals could not be uniquely identified during spawning and I could not discern if individuals spawned repeatedly in a single spawning season. Reproductive synchrony in the same local habitat is prevalent across many taxa, including mass spawning of corals, plant seeding, and spawning aggregations of fishes (Harrison et al. 1984; Kelly and Sork 2002; Koizumi et al. 2008). Reproductive synchrony within the same stream, but not among isolated streams, may be driven not only by abiotic cues but also by intra-specific interactions among individuals (Harrison et al. 1984; Bruch and Binkowski 2002; Engen and Saether 2006; Koizumi et al. 2008; Forsythe et al. 2011; Chevalier et al. 2014). Reproductive synchrony within the same streams may have evolved as an adaptive strategy to maximize offspring survival (Harrison et al. 1984; Kelly and Sork 2002), a topic which is beyond the scope of this study but warrants further investigations.

The GLARMA model was a useful framework to account for daily spawning periodicity of bluehead chub and yellowfin shiner. Unlike other time-series models using ARMA components, the GLARMA model accepts polynomial AR and MA terms that increase flexibility when analyzing complex serial data (Benjamin et al. 2003; Dunsmuir and Scott 2015). Bluehead chub and yellowfin shiner exhibited the periodic spawning pattern, but intervals of spawning events varied over the spawning season (Fig. 1-3). Further, although the peak timing was May, fish still spawned intermittently until late June (Fig. 1-2). Given these complex serial

data, the GLRMA model was capable of accounting for spawning periodicity by accepting

polynomial AR and MA terms (Table 1-2 and Fig. 1-4). Data on spawning timing of fishes are

frequently collected to estimate reproduction, larval emergence, and migration at daily, monthly,

or annual intervals (Reid 2006; Forsythe et al. 2011; Krabbenhoft et al. 2014; Catalano and

Bozek 2015; Straight et al. 2015). Several models (e.g., a generalized linear mixed model with

time-series components) have been successfully applied to address the reproductive timing when

fish spawn intensively in a short period or exhibit a consistent periodic pattern (Falke et al.

2010a Forsythe et al. 2012; Straight et al. 2015). However, if a time-series data set deviates from

a regular pattern, the GLARMA model can noticeably improve model fit. A potential caveat of

the GLARMA model lies in interpreting ecological significance of their covariates and inherent

structural components (i.e., AR and MA terms). To facilitate ecological understanding, I found it

useful to start with a simpler model (e.g., GLM) and add complexities gradually, complemented

with visualization of raw data (Fig. 1-5 and 1-6).

Nest association among freshwater fishes is common throughout North America

(Johnston 1994a). I never observed yellowfin shiner spawning solitarily without bluehead chub,

a phenomenon similarly observed by Wallin (1989; 1992). Spawning timing of nest associates

has evolved concurrently with host species, thus understanding the reproductive ecology of

keystone species is crucial for community- and ecosystem-wide conservations (Vives 1990;

Johnston 1994b; Peoples et al. 2015; Peoples and Frimpong 2016). These findings demonstrate

that daily variation in environmental variables affects spawning periodicity of bluehead chub and

their nest associate (i.e., yellowfin shiner). Consequently, anthropogenic activities and climate

impact that disrupt the daily environmental patterns may affect spawning periodicity and

population viability of multiple species (Lohse et al. 2008; Falke et al. 2010b; Krabbenhoft et al.

2014; Peoples et al. 2014; King et al. 2016). Frequent changes in stream discharge in flow-regulated rivers (e.g., hydroelectric dams) may not provide an optimal range of water depth and velocity for a sufficiently long period, preventing successful reproduction in bluehead chub (Poff et al. 1997; Peoples et al. 2014; Kemp 2017). Modified land cover (i.e., impervious surface) leads to flashier flow and thermal regimes, which causes reproductive failures (Lohse et al. 2008; Steel et al. 2015; Kemp 2017). Anthropogenic stressors are likely to act synergistically with climate change to affect spawning phenology of hosts and associates (Bestgen et al. 2006; Morisette et al. 2009; Warren et al. 2012). Knowledge of reproductive periodicity associated with environmental cues is paramount as I attempt to conserve stream fish assemblages in the face of multiple anthropogenic stressors.

Tables and Figures

Table 1-1. Habitat characteristics of each study stream.

Stream	Mean wetted width (m)[1]	Mean depth (cm)[1]	Dominant substrate[2]	Canopy cover
Indian Creek	2.8	16.6	PE, SD	High
Todd Creek	3.8	24.7	PE, CO	Low
Sixmile Creek	7.4	32.1	SD, GV	Intermediate

[1]Mean wetted width and depth were based on measurements at transects, which were evenly distributed across study sites (Indian Creek: 48, Todd Creek: 32, Sixmile Creek: 20).
[2]Dominant substrate was visually estimated at transects. SD, sand (0.01 – 2 mm); GV, gravel (2 – 16 mm); PE, pebble (16 – 64 mm); CO, cobble (64 – 256 mm).

Table 1-2. Parameter estimates of the Generalized Linear Auto-Regressive Moving Average model to predict spawning periodicity of bluehead chub in three study streams. Significant environmental covariates ($\alpha < 0.05$) are shown in bold.

Stream	Term[*]	Parameter	Estimate	SE	*Z*-ratio	*P*-value
Indian Creek	AR	1	0.482	0.071	6.757	<0.001
	AR	16	-0.432	0.110	-3.941	<0.001
	Intercept		**-1.836**	**0.437**	**-4.199**	**<0.001**
	Water temperature	**Linear**	**11.734**	**5.010**	**2.342**	**0.019**
	Water temperature	**Quadratic**	**-15.340**	**5.900**	**-2.600**	**0.009**
	Water level	Linear	3.941	2.881	1.368	0.171
	Water level	Quadratic	-0.580	1.643	0.353	0.724
Todd Creek	AR	2	0.274	0.101	2.716	0.007
	AR	4	-0.573	0.089	-6.446	<0.001
	AR	6	-0.537	0.107	-5.007	<0.001
	AR	15	0.136	0.055	2.494	0.013
	MA	1	0.712	0.040	17.998	<0.001
	Intercept		**-2.195**	**0.331**	**-6.641**	**<0.001**
	Water temperature	**Linear**	**8.887**	**2.614**	**3.400**	**<0.001**
	Water temperature	**Quadratic**	**-12.597**	**2.319**	**-5.432**	**<0.001**
	Water level	Linear	-3.746	3.770	-0.993	0.321
	Water level	**Quadratic**	**-10.206**	**4.953**	**-2.222**	**0.027**
Sixmile Creek	AR	3	-0.199	0.083	-2.401	0.016
	AR	6	-0.297	0.107	-2.777	0.005
	AR	11	-0.822	0.116	-7.101	<0.001
	MA	1	1.070	0.321	3.334	<0.001
	Intercept		**-1.455**	**0.407**	**-3.572**	**<0.001**
	Water temperature	**Linear**	**13.897**	**5.940**	**2.337**	**0.019**
	Water temperature	Quadratic	3.467	4.087	0.848	0.396
	Water level	**Linear**	**15.259**	**7.033**	**2.170**	**0.030**
	Water level	**Quadratic**	**-15.818**	**6.827**	**-2.317**	**0.021**

[*] AR, auto-regressive term; MA, moving average term.
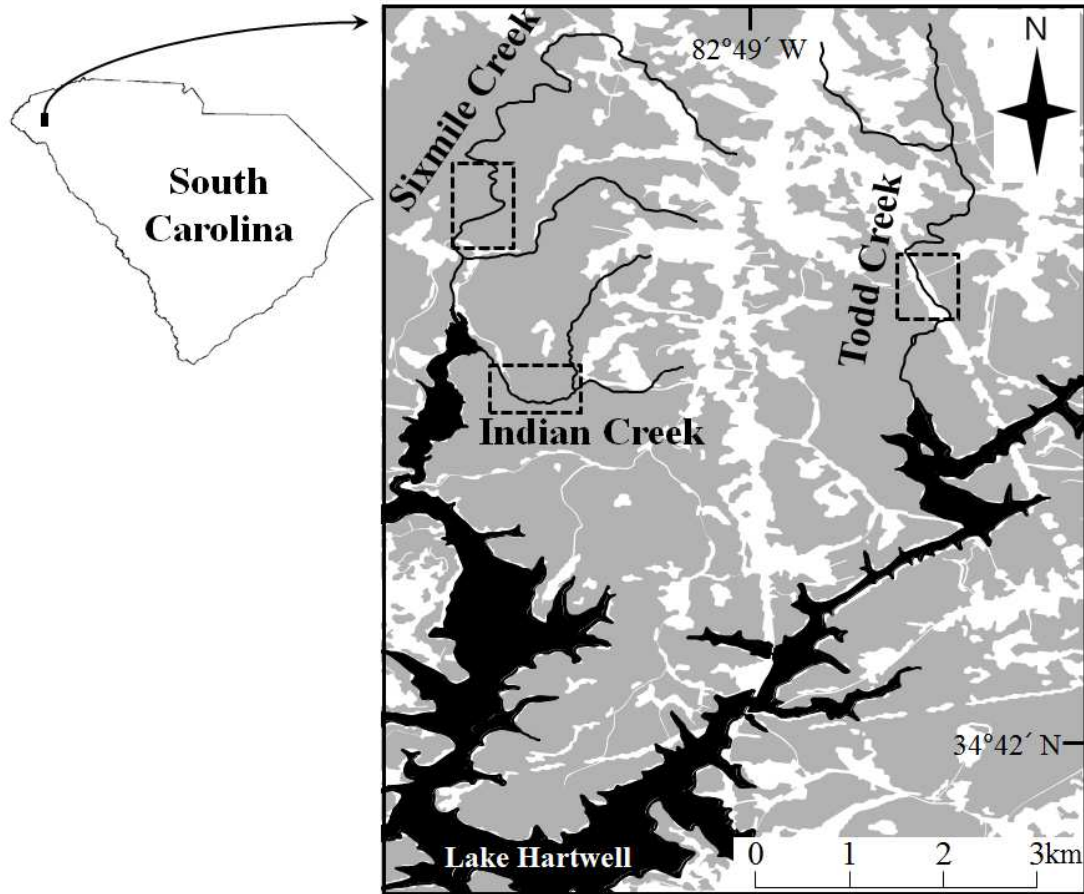
Fig. 1-1. Map of study streams in the Savannah River Basin in the upper Piedmont region of South Carolina, USA. Waterways (lakes and streams) are shown in black, forested areas are gray, and developed areas and roads are white. Dotted rectangles indicate the study area in each stream.
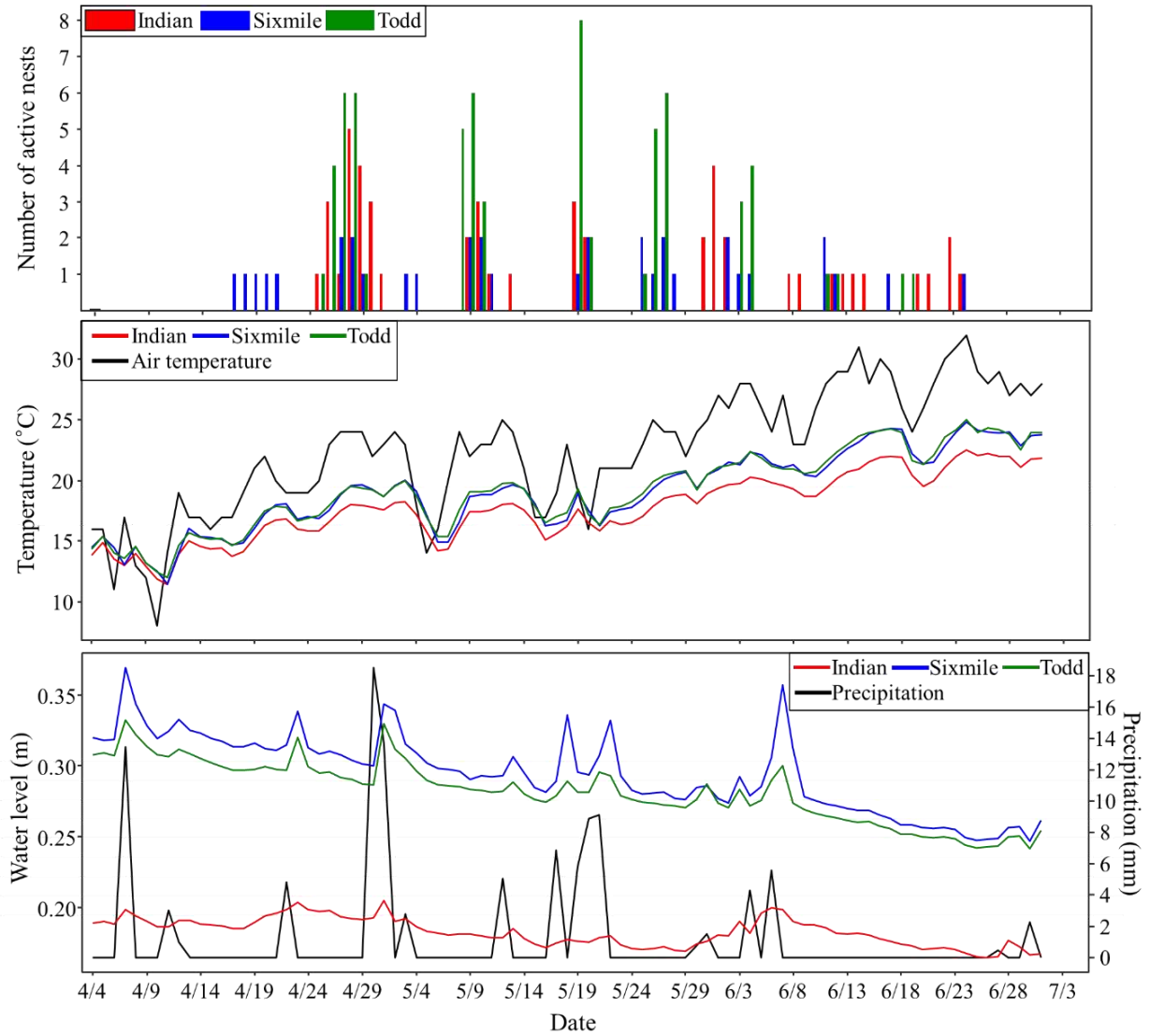
Fig. 1-2. Time series plots of the number of active nests (top), temperature (middle), and water level and precipitation (bottom) from April 4th to July 1st in 2016.
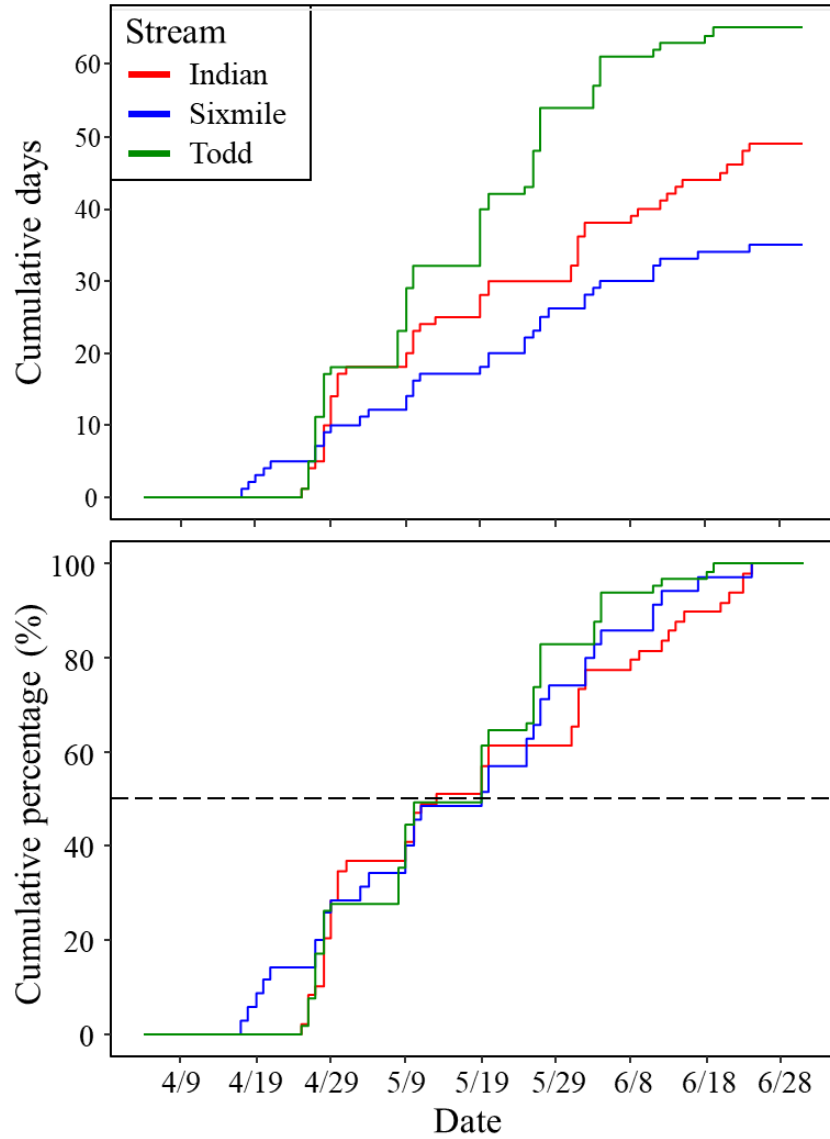
Fig. 1-3. Cumulative days (top) and percentage (bottom) of active nests in each study site during the 2016 spawning season. Cumulative days were a sum of number of days that each nest was active up to the observation date. The black dashed line indicates the mid-point (50%).
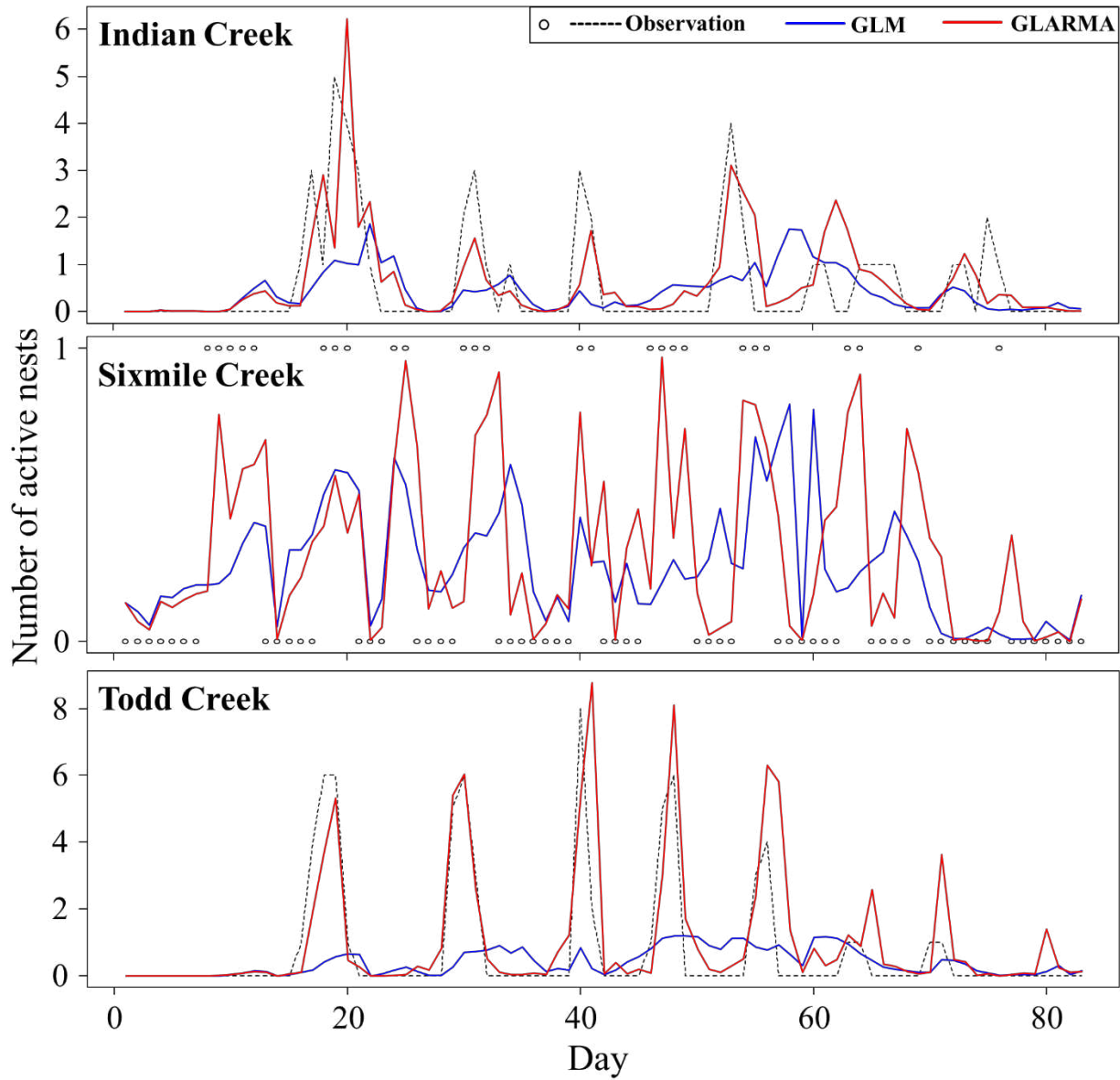
Fig. 1-4. Observed versus expected count (Indian and Todd Creeks) and presence (Sixmile Creek) of active nests using GLM and GLARMA models.
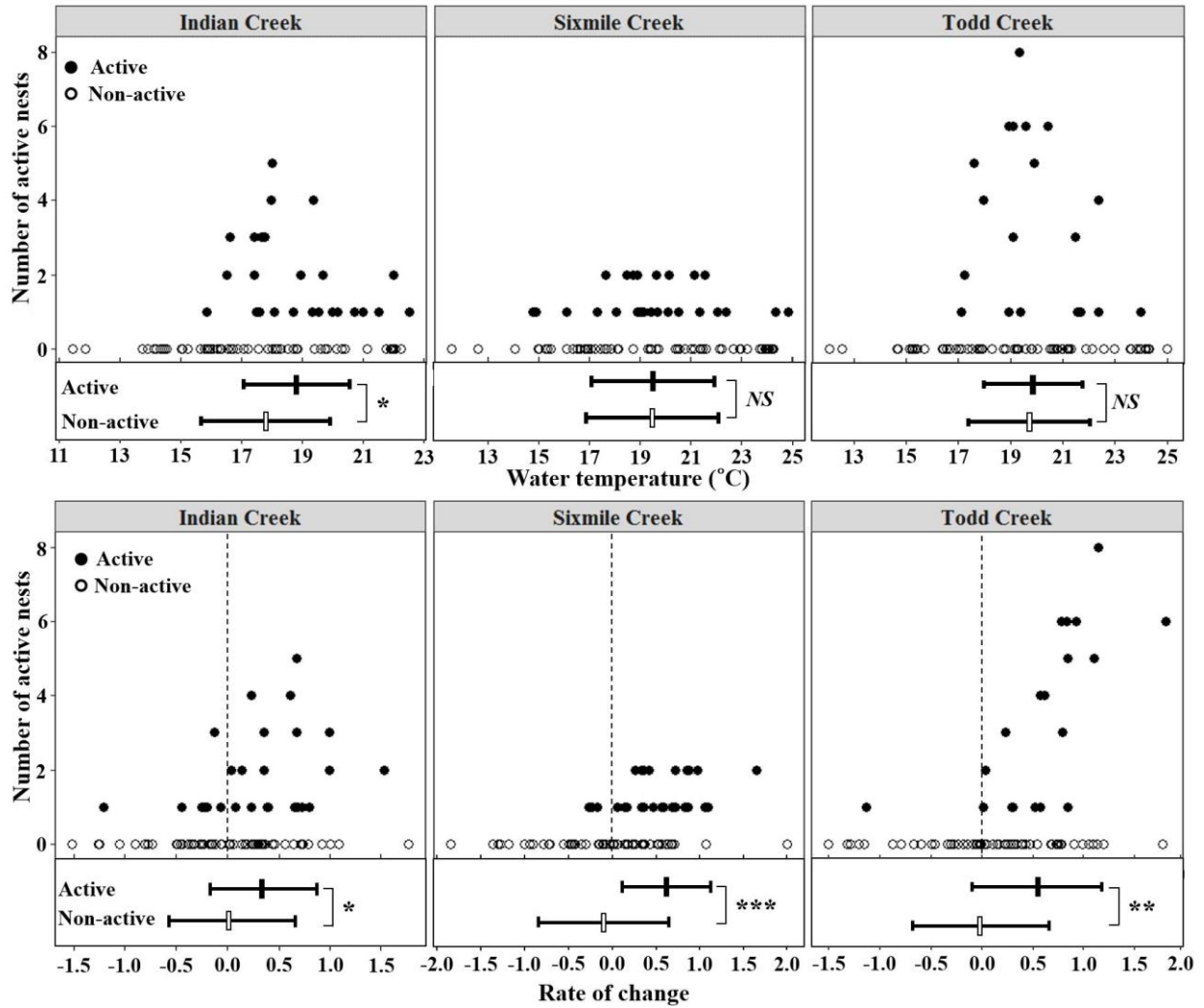
Fig. 1-5. Scatter plots showing relationships between the daily number of active nests and water temperature (top) and its rate of change over 2 days (bottom). Each scatter plot is accompanied by error bars that summarizes the mean and standard deviation on days with or without active nests with *t*-test results (*NS*, *P*-value ≥ 0.05; *, *P*-value < 0.05; **, *P*-value < 0.01; ***, *P*-value < 0.001).
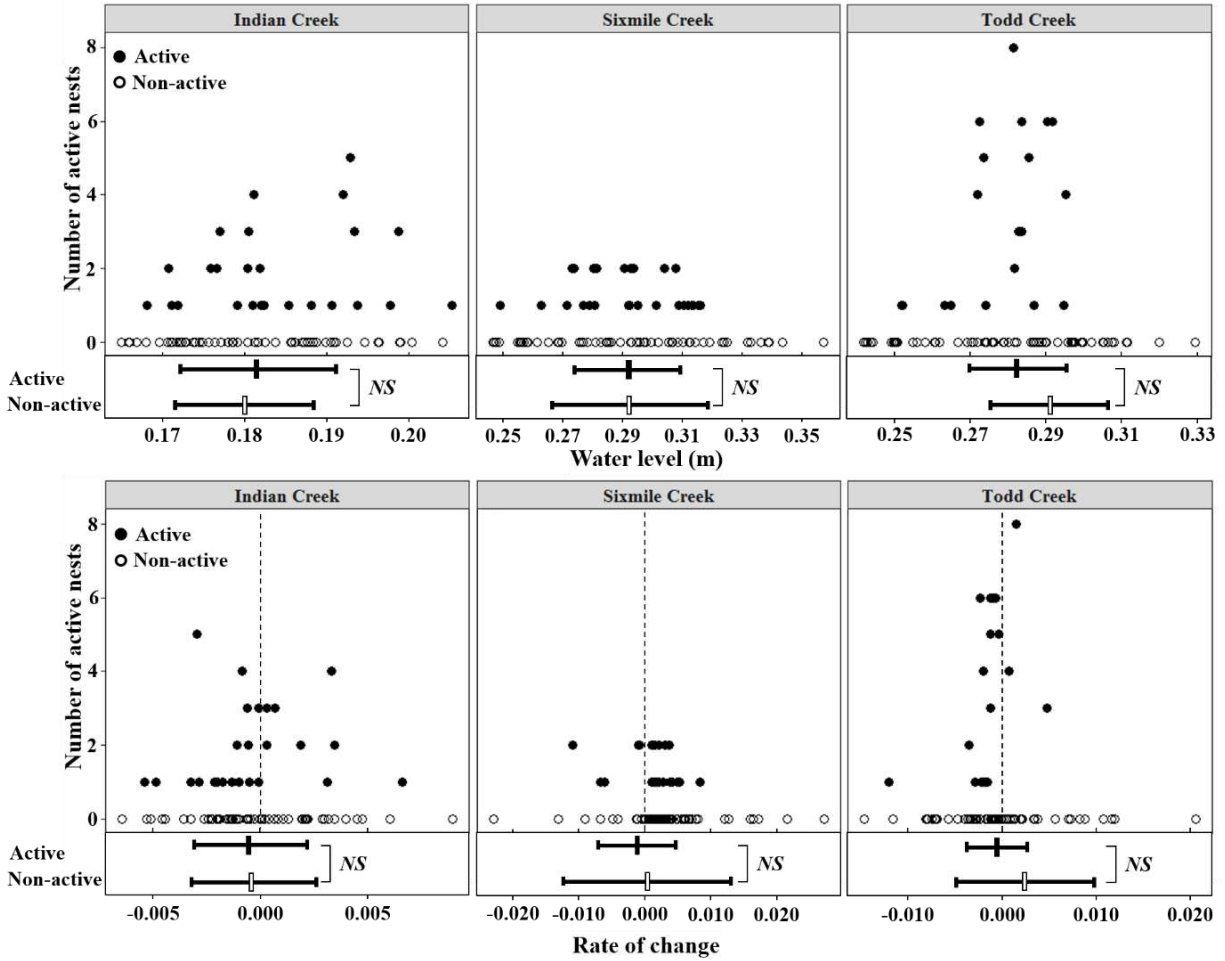
Fig. 1-6. Scatter plots showing relationships between the daily number of active nests and water level (top) and its rate of change over 2 days (bottom). Each scatter plot is accompanied by error bars that summarizes the mean and standard deviation on days with or without active nests with *t*-test results (*NS*, *P*-value ≥ 0.05; *, *P*-value < 0.05; **, *P*-value < 0.01; ***, *P*-value < 0.001).

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CHAPTER 2: MALE BODY SIZE MEDIATES NESTING BEHAVIOR OF BLUEHEAD

CHUB AND REPRODUCTIVE INTERACTIONS WITH A NEST ASSOCIATE,

YELLOWFIN SHINER

1. Introduction

Fishes exhibit an array of parental care behavior for their offspring (Balon 1975; Gross

and Sargent 1985; Gross 2005). Nest construction, a form of parental care, is widely used among

fishes (Winemiller and Rose 1992; Sinervo and Zamudio 2001). Nest construction can serve two

main functions for male fitness. First, males construct nests to protect eggs and larvae against

predation and dynamic environmental conditions (e.g., stream flow) (Bruton and Gophen 1992;

Suski and Ridgway 2007; Rushbrook et al. 2010). Thus, nest construction can maximize

offspring survival by providing shelter and a stable environment (Chin-Baarstad et al. 2009;

Rushbrook et al. 2010). Second, nests provide information on male quality to females that

choose mates (Barber et al. 2001; Blais et al. 2004). Accordingly, variation in nest size and

design can be a complex component of fish mating systems, and strongly affects reproductive

success (Wong et al. 2007; Barber, 2013). Nests may differ in location, timing, size, and

structure (Lukas and Orth 1995; Dauwalter 2007; Barber 2013). Selecting a nesting location is

key for successful spawning, but suitable nesting sites may be limited by environmental

conditions, resource availability (e.g., substrate) and population density (Rushbrook et al. 2010;

Lehtonen et al. 2015). For example, stream flow has a strong impact on physical habitat

characteristics such as water depth and velocity, as well as materials for nest construction

(Bruton and Gophen 1992; Lukas and Orth 1995; Dauwalter 2007). Limited resources can lead

to reproductive competition among males (Wong et al. 2007), with dominant males securing the best nesting sites and materials (Wiegmann and Baylis 1995; Lehtonen et al. 2007).

Male body size is an important phenotypic trait tightly associated with the timing of spawning, social hierarchy, territoriality, and nest quality; thus it can have a large impact on reproductive success (Lindström 1992; Taborsky 1994; Barber 2013). Larger males tend to spawn earlier and occupy better territories (Oliveira et al 1999; Uglem and Rosenqvist 2001). They secure nesting sites with aggressive behavior and construct higher quality (e.g., larger) nests by acquiring nesting resources first (Wong et al. 2008; Lehtonen et al. 2015). Such dominance is advantageous for not only gaining access to more females (Blais et al. 2004; Lehtonen et al. 2007), but also protecting eggs to increase offspring survival (Suski and Ridgway 2007; Chin-Baarstad et al. 2009).

However, smaller males can overcome the size-dependent disadvantage by selecting alternative reproductive strategies, such as temporal segregation and parasitic behavior (Danylchuk and Tonn 2001; Sinervo and Zamudio 2001; Taborsky 1994). Nest sharing behavior (where multiple males coordinate their behavior to attract females to the same nest) may also offset the size-mediated influence on reproductive success (Díaz-Muñoz et al. 2014). Several nest sharing behaviors have been documented in fishes (Taborsky 1994; Díaz-Muñoz et al. 2014). Males of some fishes show mutual tolerance and share mates while spawning at the same nesting sites (Reighard 1920). Cooperative parental care among individuals is also known in some fish species (Reighard 1943; Wallin 1989). Such an array of reproductive strategies provides valuable insights into reproductive cooperation among male fish, but questions still remain about how nest sharing behavior affects the quality of nests and resulting reproductive outcomes.

Little is known about how variation in size of males and nests may affect or be affected by reproductive interactions with other fishes. Positive reproductive interactions (mutualism, commensalism, and facilitation) occur between many nest-building species and 'nest associates', fishes that spawn in nests constructed by other species (Johnston and Page 1992). Nest associative behavior is diverse, having evolved independently on at least three continents among a variety of taxa (Wisenden 1999). In North America, nest association is most common among minnows (Leuciscidae). During nest associative spawning, associates often greatly outnumber nest building individuals. Eggs of associates comprise up to 97% of eggs in a nest (Wallin 1992; Cashner and Bart 2010). Accordingly, nest building species benefit from a 'dilution effect' by which high proportions of associate eggs on nests greatly reduce the probability of predation on eggs of nest building species (Johnston 1994a; Silknetter et al. 2019). Moreover, previous studies have shown that associate species select nests guarded by larger males for spawning (Shao 1997). Accordingly, understanding how male body size and nest size operate to attract associates will be critical for understanding the role these factors play for determining reproductive success of nest building fishes.

Bluehead chub (*Nocomis leptocephalus*) (hereafter, "chub") is a suitable species to study intraspecific variation in nesting behavior and interspecific interactions. Chub are distributed throughout the Atlantic slope of North America and are abundant in the Blue Ridge and Piedmont of South Carolina (Lanchner 1952; Marcy et al. 2005). Chub engage in nest associative spawning with yellowfin shiner (*Notropis lutipinnis*) (hereafter, "shiner"), which is also common throughout the region (Wallin 1989; 1992; Marcy et al. 2005) (Fig. 2-1). Nesting sites are commonly limited by availability of key mesohabitats (e.g. pool tails and slow runs; Peoples et al. 2014), microhabitats (optimal depths and velocities; Bolton et al. 2015; Peoples et

al. 2016), and/or gravel availability. Single or multiple males construct a dome-shaped nest and spawn on the nest (Wallin 1989; Sabaj et al. 2000). After spawning, males continue to move pebbles onto the nest, increasing the size of the nest (Wallin 1989; Peoples et al. 2016). Additionally, males may utilize pebbles from old nests to build new nests (Wallin 1989). Previous observations suggest that the behavioral variation (i.e., nest re-use) associated with limited nest resources is common (Wallin 1989). However, the effect of male body size on behavioral variation is poorly studied in this species group. Like other leuciscids, shiner are an obligate nest associate (Peoples and Frimpong 2016), and cannot spawn without a host in the wild (Wallin 1992). However, the factors that contribute to nest selection by shiner remain unstudied.

The goal of this study was to quantify abiotic and biotic factors affecting two key variables related to the reproductive success of chub: 1) nest size at the completion of reproductive activities, and 2) nest utilization by shiner (shiner abundance on nests). I focused on these two response variables because (1) nest size is typically an indicator of reproductive success for male fishes (Uglem and Rosenqvist 2001; Lehtonen et al. 2007), particularly in polygamous mating systems of chub where males can mate with multiple females and larger nest size can accommodate more females (Wallin 1989; Sabaj et al. 2000), and (2) higher abundance of spawning shiner benefit egg survival of chub via the dilution effect (Johnston 1994b; Silknetter et al. 2018). I developed a working conceptual hypothesis of causal relationships among natural abiotic features (photoperiod, precipitation, etc.), biotic features associated with chub fitness and spawning behavior (e.g. male size, amount of nest building, etc.), and the two variables potentially influencing chub fitness (Fig. 2-1). I then tested this set of hypotheses using the path analysis.

2. Methods

To understand how male body size affects nest size and number of mutualistic nest associates, I conducted daily observations of chub nests on three streams in northwestern South Carolina. I calculated several variables indexing male chub reproductive behavior and environmental conditions. I then developed a causal conceptual hypothesis depicting interrelationships between these variables and nest size/number of shiner (Fig. 2-2). I evaluated this hypothesis statistically with path analysis, a useful tool for quantifying complex relationships in which independent variables can also be dependent (endogenous), ultimately leading to prediction of terminal (or exogenous) variables (nest size and number of shiner).

2.1 Study area

This study was conducted at three streams in the Clemson University Experimental Forest, located in the upper Piedmont region of South Carolina, USA. Study streams were selected based on size and habitat characteristics for tractable field observations and the occurrence of focal species. Indian Creek (34°44'32"N, 82°51'05"W) was the smallest stream (mean wetted width = 2.7 m), which was covered by mixed hardwood canopy. Substrate was composed mainly of pebble (16 – 64 mm) and sand (0.1 – 2 mm), and I studied a reach of 1.6 km. Todd Creek (34°45'15"N, 82°48'56"W) was intermediate in size (mean wetted width = 3.8 m) with open canopy, where riparian area was covered by shrubs. The dominant substrate was pebble and cobble (64 – 256 mm), and I selected a 0.8 km study stream reach. Sixmile Creek (34°45'36"N, 82°51'25"W) was the largest (mean wetted width = 7.3 m) of the three streams. The stream was mainly covered by overhanging trees but some parts of the stream had little shade from riparian shrubs. Dominant substrate was sand and gravel (2 – 16 mm), and a 1.0 km

reach was studied. Common species found across all streams included bluegill (*Lepomis macrochirus*), creek chub (*Semotilus atromaculatus*), green sunfish (*L. cyanellus*), redbreast sunfish (*L. auritus*), and striped jumprock (*Moxostoma rupiscartes*). Rosyface chub (*Hybopsis rubrifrons*), another known nest associate (Marcy et al. 2005), was found in Indian and Sixmile Creek, but were much less abundant than yellowfin shiner.

2.2 Fieldwork

Field observations were conducted for 89 consecutive days from April 4th to July 1st in 2016 to record spawning activities of chub and shiner and environmental conditions. Two observers walked on the stream bank or in the stream channel to locate chub nests. Male chub prefer pool tails or run mesohabitats covered by gravel and pebble for nesting (Wallin 1989; Sabaj et al. 2000; Bolton et al. 2015), but observers checked all habitat types including runs, riffles, pools and undercut banks. Observers were confidently able to locate chub nests by their conspicuous pebble mounds. When a new nest was located, a marker was placed on the nearest bank with a unique nest identification number. Observers checked the status of marked nests, and nest dimensions (length, width, and height) were measured daily until nests disappeared due to high flows or re-usage by other males.

Shiner spawn on chub nests, during which they display brilliant breeding colors (i.e., red body color with yellow fins) (Fig. 2-1) and spawning of both species is synchronized (Wallin 1989; 1992). Thus, I considered a nest to be 'active' when more than one shiner with breeding colors were observed schooling or spawning on the nest (Table 2-1). Once an active nest was located, observers stayed on the nearest bank for at least 15 minutes and used binoculars to record the spawning activity. Male chub have conspicuous external characteristics such as

tubercles on their head and body colors (Marcy et al. 2005), making them easy to identify. I counted the number of male chub on each nest, and visually estimated their total lengths into five size groups (< 9, 9-12, 12-15, 15-18, or > 18 cm). The behavior of male chub, such as spawning, constructing the nest, and interacting with other males, was also recorded. I also visually approximated the number of shiner spawning on the nest (0, 1-10, 10-50, 50-100, or > 100 individuals).

Environmental data were collected at various spatial scales. Daily precipitation (mm) and photoperiod (minute) were gathered from the nearest weather station (Clemson-Oconee County Airport Weather Station by the National Weather Service), approximately 10 km from the study area. Water temperature (°C) and level (m) were recorded hourly by using loggers (Model U20L-004, HOBO Onset Computer Corp., Bourne, Massachusetts) at the downstream end of each study stream. Water velocity (m·s^{-1}) and depth (cm) were measured daily at four points around each nest (upstream, downstream, right, and left).

2.3 Variable calculation

We were interested in two key variables indexing chub fitness: nest size and number of shiner using the nest (Table 2-1). First, nest size was calculated as the volume of elliptical cone (m^3):

V (volume, m^3) $= \pi a b \frac{h}{3}$ (1)

where *a* and *b* were half of the length of major and minor axes, and *h* was the height of the nest. Shiner abundance, an ordinal variable, was defined as the maximum number of individuals observed during a single observation. Shiner spawn on the same nest for more than one day (Kim and Kanno in review), and different individuals could be present on different days. However,

shiner individuals could not be uniquely identified and the maximum number of shiner individuals serves as a measure of attractiveness and carrying capacity of a single chub nest.

We defined six endogenous variables indexing male nesting behavior (Table 2-1). Male size was treated as an ordinal variable. Male chub move pebbles onto their nests to protect their eggs after spawning (Wallin 1989; Sabaj et al. 2000). Thus, the number of days that the nest size increased during the active and post-spawning periods was considered a measure of nest building. Nesting locations are frequently re-used because they are generally limited by substrate supply and microhabitat conditions (Wallin 1989; Peoples et al. 2016). Three nest types were identified during the field observations (Fig. 2-3). Nests that were constructed but abandoned without signs of spawning were defined as 'abandoned'; many nests were constructed overnight and abandoned (evidenced by silt coverage and no activity) by the next morning. The nest type 'new' indicated when male chub constructed nests at new nesting sites. Otherwise, male chub frequently re-used nesting locations; this 'reused' type had two variants: (1) Pebbles from an old nest were used to construct a new nest in an adjacent location (reconstructed type), (2) The same location was used repeatedly at different times but pebbles were added to the foundation of an old nest (expanded type). The nest type 'abandoned' was excluded for the analysis because spawning was not observed. Nest type was included as a binary variable (new versus reused) (Table 2-1). Nest duration was defined as the number of days that a nest was active. Active nest size was the average size of the nest when it was active (Fig. 2-3).

Daily mean water temperature and level were calculated based on hourly measurements from a logger in each stream. Mean water depth and velocity at nests were calculated from daily measurements in the field (Table 2-1).

2.4 Conceptual hypothesis

We developed a conceptual path diagram to examine hypothesized direct and indirect

relationships among variables, where each arrow indicates hypothesized causality (Fig. 2-2).

The diagram represents two key tests with male body size playing a pivotal role in the potentially

intricate network. First, temporal spawning patterns may be explained by male body size, with

larger males spawning earlier (Lindström 1992; Oliveira et al. 1999; Uglem and Rosenqvist

2001). Chub spawn for two to three months in spring and summer, during which time

photoperiod and water temperature increase, and stream flow and water level decrease in the

study region (Wallin 1989, 1992; Kim and Kanno in prep). Abiotic variables were hypothesized

to affect the timing of spawning of different-sized males. We also hypothesized that nest re-use

would be more common later in the spawning season, because ideal nest sites used by early-

spawning males are re-used by late-spawning males (Fig. 2-2). Second, we tested how male body

size affects other biotic variables related to nest construction, and ultimately two terminal

variables of interest—maximum nest size and shiner abundance. Male size was hypothesized to

affect all other biotic variables and two terminal variables directly or indirectly, but we also

established causal pathways between other biotic variables and terminal variables (Fig. 2-2). We

hypothesized that maximum nest size was affected by variables from active and post-spawning

periods, whereas the number of shiner was only influenced by variables related to active period

(Table 2-1 and Fig. 2-2). The nest duration and active nest size could affect the maximum nest

size; however, pathways from these variables to maximum nest size were not established for

following reasons. The nest building variable included both active (i.e., nest duration) and post-

spawn periods (Table 2-1), thus nest duration was partially redundant with nest building. The

active nest size and maximum nest size were estimated by daily recorded data (Table 2-1), thus

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active nest size could be temporally correlated to maximum nest with a redundant causal relationship. Pathways from the nest type and nest building to the number of shiner were not established because shiner were not likely to distinguish among nest types, and male chub provided nest building after shiner had completed spawning.

2.5 Statistical analysis

I used path analysis to quantify factors affecting variation in maximum nest size and the number of shiner on each nest (Fig. 2-2). Path analysis is a specific case of structural equation modeling with no latent variables (Shipley 2002), and is ideal for testing complex network relationships in which variables can be both predictors and responses (Shipley 2000; Kline 2011). In producing an aggregate network of linear modes (i.e. sub-models or paths), path analysis is unique in providing inferences on specific paths (regression coefficients, *b*) as well as the global model. Path analysis also allows for the estimation of indirect effects as the product of all paths leading to a given response.

Prior to analysis, all variables were log (x+1) transformed to improve normality and standardized by mean (mean = 0 and standard deviation = 1) to allow direct comparison of effect sizes. Two dummy variables were used to account for inherent variation among three streams (one stream as a reference). Each hypothesized pathway was tested based on statistical significance set at $\alpha = 0.05$, and non-significant paths were removed sequentially, beginning with the lowest effect size, to develop a parsimonious model that passed model fit diagnostic. Path analysis was fit using robust maximum likelihood estimator (Enders 2001). The final model was evaluated based on the global goodness of fit (χ^2 test, $\alpha > 0.05$), root mean square error of approximation (REMSEA, range = 0–1, < 0.05 acceptable), and comparative fit index (CFI,

range = 0–1, > 0.9 acceptable) (Vile et al. 2006). Failing to reject the global null hypothesis (χ^2) indicates that the observed and expected covariance structure do not differ, indicating adequate model fit. The final model contained at least five times fewer paths than the number of observations (Petraitis et al. 1996). Analyses were conducted in the R version 3.5.1 (R Development Core Team 2018) and the path analysis was fit using the package *lavaan* version 0.6-2 (Rosseel 2012).

3. Results

During the study period (April 4th - July 1st, 2016), mean water temperature in Indian Creek (mean = 17.7°C) was typically colder than Sixmile Creek (mean = 19.1°C) and Todd Creek (mean = 19.2°C). The mean photoperiod was 833.2 minutes and increased gradually until early June and then remained stable until July 1st. Precipitation occurred on 18 days, during which the mean value was 6.01 mm. Water level declined generally during the study period but was influenced by precipitation. Mean water depth and velocity at nests were 28.71 cm (SD= 11.22) and 0.16 m·s^{-1} (SD= 0.05), respectively (Table 2-1).

A total of 92 nests were located across three study streams, and spawning was observed on 71 nests (Indian Creek: 21, Sixmile Creek: 16, Todd Creek: 34: Table 2-2). Of possible nest types, 35 nests were recorded as 'new', 36 were 'reused', and 21 were 'abandoned' (Table 2-2; Fig. 2-3). The mode of daily number of shiner on active nests was 10 – 50 individuals. Mode of male size was 15 – 18 cm. Most nests were tended by only one male (median = 1), but some were tended by up to four. Nests were active for a median of two days (range = 1 – 4) and the mean active nest size was 0.015 m^3. Male chub tended nests for a median of 7 days (range = 2 – 13). The mean maximum size of the nest was 0.026 m^3 (Table 2-1).

The global path model fit the data poorly (χ^2_{83} p= <0.001; CFI= 0.56; RMSEA = 0.18), and contained numerous uninformative paths (Appendix B1). After removing five uninformative paths, beginning with the weakest effect size, we identified a stable final model with adequate global fit and contained all significant paths. The final path model provided a good fit to the data (χ^2_{33} p= 0.66; CFI > 0.99; RMSEA < 0.001), and retained 14 paths (Fig. 2-4).

Male body size was related to the timing of spawning as mediated by environmental factors, and body size influenced spawning behavior. Water temperature increased with photoperiod (b= 0.75), and had a negative effect on male size (b= -0.37), indicating that larger males spawned earlier than smaller males (Fig. 2-5). Instances of nest reuse increased with photoperiod (b= 0.24), meaning that males frequently re-constructed nests later in the spawning season. Male size had direct and positive effects on all biotic variables, suggesting that male size was a determinant of spawning behavior. Larger males were more likely to spawn together (b= 0.40), spawn longer (nest duration, b= 0.30), attend nests longer (nest building, b=0.41), and use existing nest resources for nest construction (nest type, b= 0.12).

For the terminal variables, the maximum nest size was directly and positively affected by the number of males (b= 0.50), nest reuse (b= 0.46), and nest building (b= 0.22). The number of shiner was directly and positively influenced by the nest duration (b = 0.30) and the number of males (b= 0.40). Male size indirectly influenced both terminal variables through several pathways (Table 2-3). Male size influenced the number of male chub, nest building, and nest type, all of which had positive effects on the maximum nest size (total indirect effect= 0.35) (Table 2-3). Likewise, male size positively affected male number and nest duration, both of which had positive effects on the number of shiner (total indirect effect= 0.25) (Table 2-3). As a

result, the total effect of male size was 0.55 (direct= 0.20, indirect= 0.35) on the maximum nest size and 0.46 (direct= 0.21, indirect= 0.25) on the number of shiner (Table 2-3).

4. Discussion

Male chub size affected maximum nest size and the number of shiner by positively influencing various biotic and behavioral factors. Larger males spawned longer (i.e., nest duration), invested more effort to care for their nests, and tended to share the same nest with other males. Increased reproductive effort led to larger maximum nest size and more shiner on the nest, the latter of which is a correlate of chub's reproductive success (Silknetter et al. 2019). Notably, the total effect (direct and indirect) of male size on the two terminal variables was larger than any other single explanatory variable in the path analysis. Findings of this study corroborate previous studies that identified male body size as a key factor in the chub's reproductive system (Jenkins and Burkhead 1994; Peoples et al. 2011). We found little evidence that smaller males overcame the size-dependent disadvantage through alternative behavior (Danylchuk and Tonn 2001; Sinervo and Zamudio 2001; Taborsky 1994), except perhaps that temporal segregation of spawning by body size could potentially be a mechanism that allows smaller males to reproduce in the study system.

The number of males sharing a nest and nest re-use had the strongest direct effects on maximum nest size. Male chub invest a major proportion of energy to care for their eggs by moving thousands of pebbles after spawning (Wallin 1989; 1992). Thus, building nests together and sharing nests may save their individual effort. Male chub prefer pool tails or shallower run habitats covered by gravel and pebble as a nesting site (Bolton et al. 2015; Peoples et al. 2016), and the range of water velocity is also important for depositing eggs (Maurakis et al. 1992).

Thus, suitable nesting sites may be limited especially when gravel and pebble substrate is scarce (McManamay et al. 2010; Peoples et al. 2014; Dovel et al. 2015; Kemp 2017). This resource limitation could explain why instances of nest re-use increased over time in the spawning season. Re-using nesting sites and pebbles from old nests can similarly be considered a behavioral strategy to minimize energy expenditure. The reconstructed nest type was similarly observed by Wallin (1989). I further identified that males used old nests without re-constructing anew but adding pebbles on top of them (i.e., expanded type). This behavior would also be beneficial for male chub by saving energy to construct nests (Lindström 1992).

The role of nest construction has been mainly described as a means to attract mates and care for young (Balon 1975; Gross 2005; Sinervo and Zamudio 2001), but many studies have also shown nest association can benefit nest-building species (Goff 1988; Johnston 1994b; Peoples and Frimpong 2016; Silknetter et al. 2019). These results suggest that male body size and nesting behavior can be another key factor for attracting mutualists. Based on total direct and indirect effect sizes, male body size was the most important variable in attracting more shiner. Other significant factors, including the number of males sharing the same nest and the number of days the nest was active (nest duration), were influenced by male size and served to attract more shiner to nests. On the other hand, the size of the nest while it was active was not significantly correlated to the number of shiner. This indicated that the large size of the nest alone was not sufficient to attract more shiner, but the number and quality (i.e., body size) of male chub are likely more important determinants of shiner abundance (Shao 1997). This finding is similar to that of an experiment by Wallin (1992) who found the presence of male chub was more important than nest characteristics; shiner avoided nests which male chub stopped attending or artificially constructed nests. Just like shiner eggs afford a dilution effect to chub, reducing the

likelihood of predation on chub eggs, shiner similarly benefit by laying their eggs where more chub eggs are present, which would favor a reproductive system where both species spawn with each other.

Multiple male chub spawned on the same nest, confirming observations by Wallin (1989) and Sabaj et al. (2000). The path analysis indicated that larger males were more likely to spawn together, which was surprising given large males were observed to be aggressive with each other in previous studies (Maurakis et al. 1991; Maurakis and Woolcott 1996). Limited nesting habitat may explain why males spawned together. Nesting sites may have been limited because larger males spawned earlier than smaller males and the peak of spawning occurred in mid-April to May. Limited resources may have necessitated larger males to share a nest and spawn together. I further observed that nest sharing involved a social hierarchy among males. Specifically, the largest male usually dug a pit for spawning at the front area of a mound (presumably the most ideal location), and other males made their own pits at other areas, as observed by Maurakis et al. (1991) and Sabaj et al. (2000).

Water temperature was the only environmental variable associated with the timing of spawning of different-sized males in the path analysis; larger males spawned earlier than smaller males (Fig. 2-5). This finding is not surprising because larger males usually initiate spawning earlier than smaller males in many freshwater fishes (Lindström 1992; Uglem and Rosenqvist 2001). In addition, smaller males may not be mature enough in the early spawning season, thus leading them to spawn later (Oliveira et al. 1999). Earlier spawning may be advantageous not only for securing more suitable nesting sites and accessing more females (Noltie and Keenleyside 1987; Dupuis and Keenleyside 1988), but also for increasing survival of offspring because larger body size resulting from earlier emergence reduces the risk of predation and

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cannibalism (Conover et al. 2003; Bestgen et al. 2006; Divino and Tonn 2007). Other environmental variables, such as water depth and velocity, did not affect spawning behavior. Nesting sites may not depend on body size because male chub have a distinct preference for nesting habitat characteristics (Bolton et al. 2015; Peoples et al. 2016). Additionally, male chub frequently re-used old nesting sites, thus males of different sizes used the same nest locations during the spawning season.

This study was based on detailed observations of chub nests and spawning activities during the entire spawning season, but it did have some shortcomings. First, I could not quantify the number of female chub spawning on each nest. The number of females attracted to a nest would have characterized male fitness more directly (Lindström 1992; Barber, 2013), but I did not feel confident about enumerating the number of females on a nest without monitoring the nest continuously over the period when the nest was active. Second, I used maximum nest size and number of shiner as surrogates of male reproductive success, but I did not directly measure reproductive success (e.g., number of offspring that hatched successfully). Further research is warranted to link reproductive behavior to fitness of both sexes of chub, which will require unique identification of individuals and an experimental approach to examining reproductive success of both chub and shiner.

Tables and Figures

Table 2-1. List and description of variables included in the path analysis used to describe reproductive behavior of male bluehead chub and their interactions with yellowfish shiner. Variables were included in the conceptual diagram (Fig. 2-2) and final path analysis (Fig. 2-4).

Variables		Mean (SD) / Mode	Descriptions
Terminal Variables	Maximum nest[1] size	0.026 (0.027)	Maximum nest size after spawning/construction
	Number of shiner[2]	10 – 50	Maximum daily number of yellowfin shiner on active nest (ordinal variable)
Biotic Variables	Male size[2]	15 – 18	Maximum male length (cm) on active nest (ordinal variable)
	Male number[1]	1.57 (0.91)	Number of male chub on the active nest
	Nest building[1]	7.39 (2.20)	Days that nest size increased during active and post-spawn periods
	Nest type	NA	New or re-used nest (binary variable)
	Nest duration[1]	2.06 (0.88)	Days nest was active
	Active nest size[1]	0.015 (0.019)	Average nest size while active
Abiotic Variables	Photoperiod[1]	833.17 (34.14)	Day length (minute)
	Precipitation[1]	6.01 (5.04)	Daily precipitation (mm)
	Water temperature[1]	18.67 (3.14)	Mean daily water temperature per stream (°C)
	Water level[1]	0.25 (0.05)	Mean daily water level per stream (m)
	Depth[1]	28.71 (11.22)	Mean water depth at nest (cm)
	Velocity[1]	0.16 (0.05)	Mean water velocity at nest (m·s^{-1})

[1]Mean and standard deviation (SD).
[2]Mode.

Table 2-2. Summary of nest types located in study streams.

Study site	Abandoned	New	Reused		Total number of active nests
			Reconstructed	Expended	
Indian	11	12	5	4	21
Sixmile	7	8	1	7	16
Todd	3	15	13	6	34
Total	21	35	19	17	71

Table 2-3. Indirect effects of male size on the maximum nest size and number of shiner in the path analysis. The indirect effect was calculated by the product of all paths from the male size to terminal variables (i.e., maximum nest size and number of shiner), and total indirect effects were a sum of indirect effects.

Variable	Path	Indirect effect
Maximum nest size	Male size → Male number → Maximum nest size	0.20
	Male size → Nest building → Maximum nest size	0.09
	Male size → Nest type → Maximum nest size	0.06
Total		0.35
Number of shiner	Male size → Male number → Number of shiner	0.16
	Male size → Nest duration → Number of shiner	0.09
Total		0.25

Fig. 2-1. Photos of male bluehead chub (*Nocomis leptocephalus*) (top left) and yellowfin shiner (*Notropis lutipinnis*) with breeding color (top right), yellowfin shiner schooling on a chub nest (bottom left), and spawning of bluehead chub (bottom right). Photo credit: S. Kim.
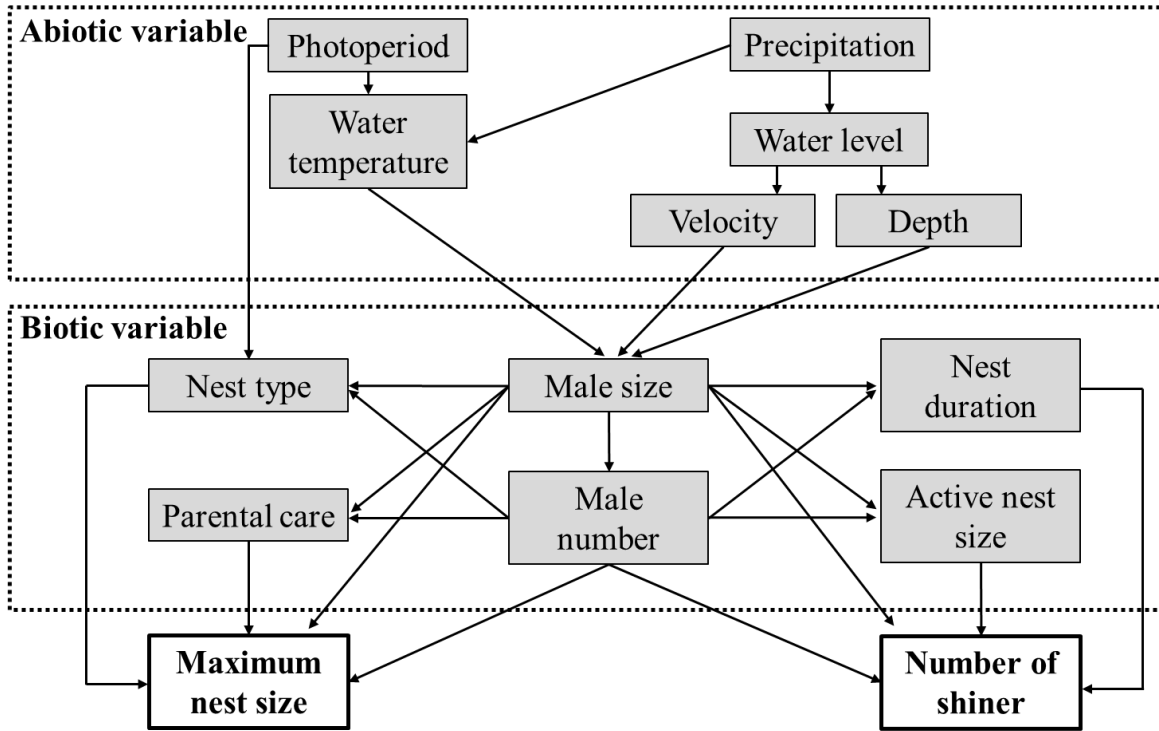
Fig. 2-2. A conceptual pathway diagram representing hypothesized relationships between abiotic and biotic factors (gray rectangles) and terminal variables (hollow rectangles) in the path analysis. Each arrow indicates hypothesized causality.

Nest type

1. Abandoned

Onset → Abandoned

2. New

→ Spawned
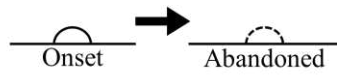
3. Reused

(1) Reconstructed

→ → Destroyed → → Newly Constructed

(2) Expanded

→ → Expanded
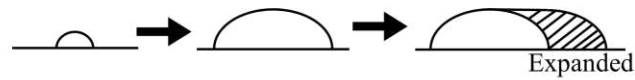
Fig. 2-3. Description of three nest types defined in this study. The re-used nest type consisted of two variants.
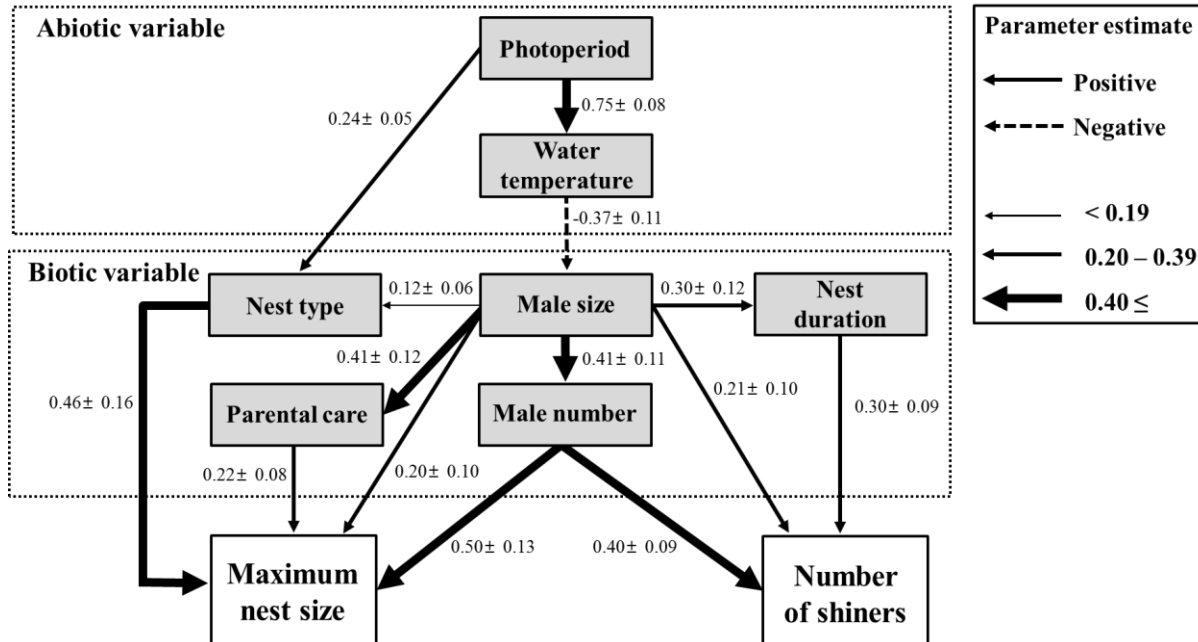
Fig. 2-4. The final path model. All arrows are significant (*P*-value < 0.05). Each arrow is shown with mean coefficient ± standard error and the thickness of arrows indicates the strength of the coefficient. Solid and dashed arrows indicate positive and negative relationships, respectively.
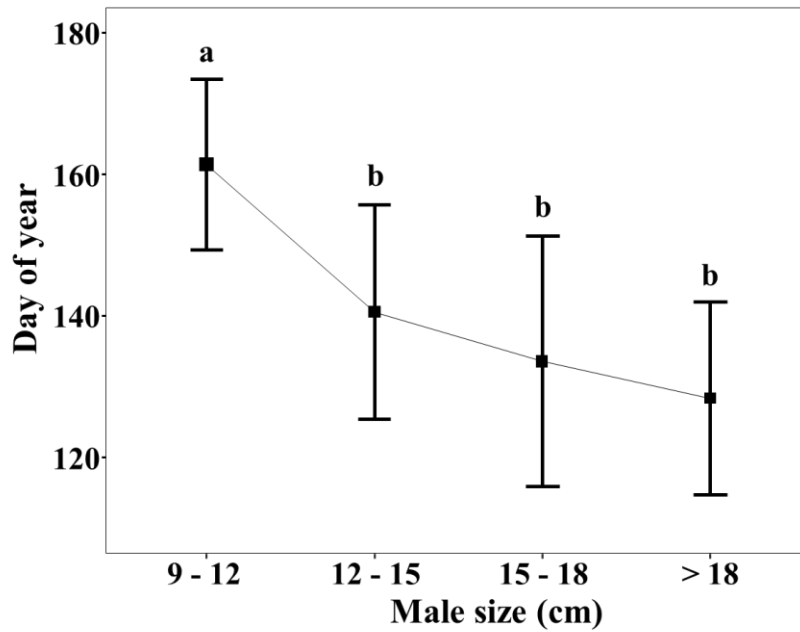
Fig. 2-5. Variation in the timing of spawning based on male chub body size classes. Black squares indicate the mean day and error bars indicate the standard deviation. Different alphabetic letters indicate means are significantly different (*P*-value < 0.05) based on the Tukey's honestly significant difference *post-hoc* test following a one-way analysis of variance.

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CHAPTER 3: INDIVIDUAL VARIATION AND NEST QUALITY AS FACTORS

AFFECTING MATING SYSTEM AND REPRODUCTIVE SUCCESS IN A NEST-

BUILDING FISH, BLUEHEAD CHUB

1. Introduction

Reproduction is a determinant of life history, population and genetic structures, and

fitness (Andersson 1994; Brommer 2000; Sinervo and Zamudio 2001). Thus, how individual

variation affects mating system and reproductive success is a classic question in animal ecology

and evolution (Reynolds 1996). A wide variety of factors have been identified to contribute to

individual variation, including body size, behavior, and resource holding (Oliveira et al. 2000;

Serbezov et al. 2010; Lehtonen et al. 2015), and these individual traits are associated with

population structures and environmental conditions (Lichstein et al. 2007; Niemelä et al. 2013).

However, the relative importance of individual traits in driving reproductive success remains

elusive, and our limited knowledge comes from a subset of taxa that are comparatively easy to

observe in the wild or laboratory experiments (Bose et al. 2018).

Nest construction is a distinct reproductive strategy that has evolved to maximize fitness,

and it is prevalent in a variety of taxa, particularly in birds and fishes (Barber 2013). Nesting is to

maximize offspring survival by providing shelter against predation risk and environmental

fluctuations (Suski and Ridgway 2007; Chin-Baarstad et al. 2009). Since nest construction is

energetically demanding (Blais et al. 2004; Mainwaring et al. 2014), the quality of nest is an

honest indicator of male quality and physiological condition (Lehtonen et al. 2007; Bose et al.

2018). Females are commonly known as the "choosy sex" (Andersson 1994) and display mating

preference by assessing male quality such as resource holding potential (e.g., territory and nest)

(Brooks and Couldridge 1999; Lehtonen et al. 2007). Thus, female choice coupled with nest quality may affect reproductive outcomes and mating systems (Barber 2013; Bose et al. 2018).

Body size is a primary individual trait that strongly and positively affects reproductive success in both sexes (Basolo 2004; Johnson and Hixon 2011). Larger males spawn earlier (Oliveira et al 1999), occupy better nesting resources (Wong et al. 2008; Lehtonen et al. 2015), and display aggressive behavior to protect nests and mates (Gross and Sargent 1985). Consequently, larger males gain access to more females and increase offspring survival through better resource holding (Blais et al. 2004; Lehtonen et al. 2007). Body size also plays an important role in reproductive outcomes of females due to an exponentially positive relationship between body size and fecundity in many taxa including reptiles (Cox et al. 2003), insects (Honek 1993), and fishes (Wootton 1979; Trippel et al. 1997). Thus, larger females generally have higher reproductive success (Honek 1993; Cox et al. 2003). Accordingly, body size has been used a strong indicator of individual quality associated with other traits (e.g., dominance, resource holding) and strongly affects reproductive outcomes (Garant et al. 2001; Basolo 2004; Johnson and Hixon 2011).

Although nesting and body size are key components of successful reproduction (Andersson 1994), males can optimize their reproductive success by selection alternative reproductive tactics (ARTs), especially cooperative behavior (Taborsky 1994; Gross 1996; Sinervo and Zamudio 2001). Male-male cooperation occurs when its benefits outweigh costs of competition (Clutton-Brock 2002), and males exhibit various types of cooperation to attract more females and increase offspring survival (Díaz-Muñoz et al. 2014). For instance, a cooperative coordination by multiple males is advantageous for attracting more females (Taborsky 2001). Several males share territories while they display mutual tolerance and share mates (Gross 1996).

Multiple males participate cooperatively in brood care to maximize offspring survival (Taborsky 1994). Further, unrelated males engage in cooperative serves as co-breeders (Clutton-Brock 2002), indicating that male-male cooperation is to increase their own reproductive outcomes (i.e., direct fitness) not indirect or inclusive fitness as a helper or satellite (Tarbosky 2001). Since cooperative males still compete for fitness (Clutton-Brock 2002), not all males gain equal benefits (e.g., mating and reproductive success) that can cause reproductive skew even within a cooperative male group (Taborsky 2009; Díaz-Muñoz et al. 2014). Complex relationships between males have an important impact on the evolution of individual traits, but questions still remain about how cooperative behaviors can affect individual reproductive outcomes, potentially even overcoming the advantage of large body size.

Quantifying individual traits is challenging in the wild due to the cryptic reproductive behavior or aggregation of multiple individuals in a small breeding area. Field and laboratory experiments have been widely conducted to examine factors affecting reproductive success (Lehtonen et al. 2015; Peoples and Frimpong 2016; Silknetter et al. 2019). However, these approaches could not disentangle multiple individual traits and their interactions affecting reproductive outcomes in the natural system (Bose et al. 2018). To overcome this difficulty, physical tagging of individuals would be a useful tool for identifying and tracking unique individuals over time and space (Muir et al. 2001). Passive integrated transponder (PIT) tags have been widely applied to examine growth rates (Hayes et al. 2008), movement patterns (Muir et al. 2001), and habitat selection (McEwan et al. 2011), especially in fisheries research. Such individual tagging and tracking techniques coupled with genetic parentage analysis may be useful to quantify individual traits and provide information on the role of individual variation in reproductive success.

Bluehead chub (*Nocomis leptocephalus*) is a suitable species that provides a unique system of spawning behavior and conspicuous nest construction, which may aid understanding of the individual traits affect reproductive success. Bluehead chub are known as a keystone species because males move pebbles to construct dome-shaped pebble nests as a host species (Wallin 1989; Sabaj et al. 2000), and other species (i.e., nest associates) spawn on nests alongside bluehead chub (Jenkins and Burkhead 1994; Johnston 1994). This interaction (i.e., nest association) is mutually beneficial because bluehead chub provide parental care to nest associates, which in turn provide protection from predation via a dilution effect through high proportions (up to 97%) of associate eggs in nests (Johnston 1994; Cashner and Bart 2010). Such nest construction and reproductive interactions facilitate observations of spawning behavior. Nests are occupied by a single or multiple male bluehead chub (Wallin 1989; Sabaj et al. 2000). Typically, the dominant male (i.e., largest male) secures a site for his nest and constructs the nest, while other males simultaneously spawn with the dominant male or they temporally segregate access to nests (Wallin 1989; Sabaj et al. 2000). Such nest sharing of male bluehead chub is a unique cooperative behavior because males in other nest-building fishes usually occupy and defend their own nest (Lehtonen et al. 2007; Barber et al. 2001; Bose et al. 2018). In addition, female bluehead chub spawn on a nest by approaching the rear of a male, who curves his body toward female (called "spawning clasp") to induce her to deposit her eggs into a nest (Sabaj et al. 2000), but little is known about female mate choice. Diverse reproductive behaviors, such as nest sharing, social hierarchy, and mate choice, suggest that reproductive success could vary because of individual traits. Previous studies used field experiments to characterize interspecific reproductive interactions between bluehead chub and nest associates (Wallin 1992; People and Frimpong 2016; Silknetter et al. 2019). However, these studies did not measure

intraspecific variation (i.e., individual traits) in reproductive success, and questions still remain about how reproductive outcomes are influenced by phenotypic traits. An integrated study, coupling individual marking techniques (i.e., PIT tag) and genetic parentage analysis could provide novel insights into behavioral and phenotypic diversity in fitness consequences of a cooperative nest-building fish.

In this study, I conducted monitoring in the natural system to characterize the reproductive ecology of bluehead chub. The aim of this study was to (1) characterize the mating system of bluehead chub, (2) examine individual traits (i.e., body size, nesting behavior, quality of nest, mate choice, etc.) as factors affecting reproductive success of male and female, and (3) assess which of those individual traits lead to successful nest construction. First, I hypothesized that male body size would be a determinant of reproductive success, but other individual traits such as cooperative behavior (i.e., nest sharing) may mediate the size-dependent reproductive success. Second, I also hypothesized that female reproductive success is also associated with body size, but behaviors could mediate this relationship. Lastly, I expected some key individual traits such as body size and cooperative nest sharing lead to successful nest construction and attracting more females, which could influence reproductive success (Lehtonen et al. 2007).

2. Methods

To understand individual traits affecting reproductive outcomes of bluehead chub, I conducted a field monitoring in a natural system in South Carolina, USA. Individual tagging and tracking data were collected to define and quantify individual traits, such as nest size, nest sharing, spawning frequency, and movement (Table 3-1). I then characterized mating system and

evaluated individual variation in reproductive success. Lastly, I examined how nest construction and female choice could be linked to male traits.

2.1 Study area

I conducted this study at Shoal Creek (34°48'12" N, 82°47'02"W) located in the Savannah River Basin in the upper Piedmont region of South Carolina, USA (Fig. 3-1). Shoal Creek was a second-order perennial stream (mean wetted width= 3.1 m) covered by mixed hardwoods canopy. The study site was characterized by a series of riffle-pool sequences with pebble and cobble substrate in riffles and runs and silt and sand in pools. The length of the study site was approximately 880 m.

2.2 Field methods

I conducted Capture-Mark-Recapture (CMR) sampling twice before the spawning season (January and April), and three times during the spawning season between May and June in 2017. Spawning activities of bluehead chub were not affected by fish sampling and handling in previous studies (Peoples and Frimpong 2016; Silknetter et al. 2019). Bluehead chub display a periodic spawning pattern in the spawning season (Wallin 1989; Kim and Kanno in press), and I avoided sampling when fish were spawning. Permanent 20-m sections were established in a continuous manner and fish were sampled in an upstream direction by backpack electrofishing units (Smith Root Model LR-24, Vancouver, Washington; and Halltech Aquatic Research Inc. Model HT-2000, Ontario, Canada) using a two-pass depletion approach. Electrofishing units were operated with 300-400 Volt and 30-60 Hz with DC or pulsed-DC settings. All captured bluehead chub were identified to sex and measured for total length (mm) and weight (g). Sex

was identified based on male bluehead chub's conspicuous external characteristics such as tubercles on their head and body colors (Marcy et al. 2005). However, females and immature males could not be confidently distinguished, thus individuals without males' characteristics were recorded as "potential females." Bluehead chub ≥ 60 mm in total length were tagged with 12-mm Half Duplex (HDX) PIT tags (Oregon RFID, Portland, Oregon). Fish were tagged by making a small ventral incision between pectoral and pelvic fins to insert tag into the body cavity. Incisions were kept minimal, approximately equal to the diameter of the tag. Genetic samples were taken from anal fins. Length, weight, sex, and PIT tag identification number were recorded on an Allegro2 field computer (Juniper Systems, Inc., Logan, Utah) using DataPlus software (Data Plus, Inc., Chelmsford, Massachusetts). Tagged fish were allowed to recover until normal swimming behavior was observed. A total of 686 unique individuals (64 males and 622 potential females) were tagged across CMR samplings.

Field observations were conducted from April 1st to July 12th in 2017 between 9 am and 6 pm. Observers walked on the bank to locate bluehead chub nests. Nests were confidently identified because male bluehead chub construct conspicuous pebbles mounds in shallower runs (Wallin 1989; Sabaj et al. 2000; Bolton et al. 2015). However, observers also checked other habitat types including runs, riffles, pools and undercut banks. Bluehead chub commonly engage in nest association with yellowfin shiner (*Notropis lutipinnis*) throughout the Savannah River Basin (Marcy et al. 2005) and spawning of both species is synchronized (Wallin 1989; 1992). Thus, I recorded an "active nest" when yellowfin shiner with breeding color (i.e., red body color with yellow fins) were schooling or spawning on the nest (Fig. 3-2b).

To monitor bluehead chub on active nests, I designed a loop-shaped PIT tag antenna to surround nests (Fig. 3-2a). PIT tag antenna systems (Multi-Antenna HDX Reader and manual

tuner, Oregon RFID, Portland, Oregon) were used to operate loop-shaped PIT tag antennas. The loop-shape antennas were made by four loops of AWG10 wire (diameter= 5.26 mm^2), and the diameter of antennas was 60 cm. This antenna design allowed me to maintain a consistent antenna inductance range (40 – 60 μH), which prevented a potential blind spot inside of antennas. Pebbles were attached around antennas using zip ties as an anchor on the substrate. Antennas were connected to the tuner and reader to record the detection of unique individuals (Fig. 3-2a). The power source was a 12-volt deep cycle battery (SRM-4D, Interstate Batteries, Dallas, Texas). In total, 3 readers and 6 tuners with 6 antennas were used for monitoring, thus I was able to monitor 6 nests at the same time.

When an active nest was located, a marker was placed on the nearest bank with a unique identification number before I installed loop-shape PIT tag antennas. The shape of antennas (either round or oval) was modified based on the size and shape of nests. After I installed antennas around nests, the performance of antennas was assessed to avoid a blind spot. Bluehead chub spawn over 1 – 4 consecutive days on active nests (Kim et al. in review), thus I operated antennas during the active period. Additionally, observers stayed near the active nest and recorded the number of male bluehead chub and their behaviors, such as spawning, constructing, and interacting with other males. When a nest was located in a riffle and difficult to observe, a waterproof camcorder (Sony HRDAS300/W Action Cam Underwater Camcorder, Sony Inc.) was placed in front of the nest to record fish behavior. In addition, nest dimensions (length, width, and height; m) were measured daily until they were destroyed by high flows or male bluehead chub stopped attending nests.

To measure reproductive success, young-of-the-year (YOY) bluehead chub were collected using backpack electrofishing units with a three-pass depletion approach between

August 25th to September 10th in 2017. To increase sampling efficiency of small-bodied fish, I

used a small (10-cm diameter) copper anode ring (Copp and Garner 1995). All captured YOY

were measured for total length and genetic fin clips were collected for genetic analysis. A total of

326 YOY (total length: mean= 31 mm, range= 16 – 49) were collected.

Daily precipitation (mm) was gathered from the nearest weather station (Clemson-

Oconee County Airport Weather Station by the National Weather Service), approximately 12 km

from the study area. A logger (U20L-004, HOBO Onset Computer Corp.) was deployed at the

middle stream section to record water temperature (°C) and water level (m) hourly.

2.3 Genetic analyses

I used a set of 8 polymorphic microsatellite loci (Cushman et al. in press) to conduct

genetic parentage analysis. Genetic analyses were conducted at the Hollings Marine Laboratory

in Charleston, South Carolina. Laboratory protocols are detailed in Cushman et al. (in press), and

summary genotype data can be found in Appendix C2.

A total of 64 males, 277 potential females detected on PIT antennas, and 326 YOY were

genotyped. I conducted a parentage analysis using Program COLONY 2.0.6.5 (Jones and Wang

2010). The parameter settings for the parentage assignment in COLONY2 were polygamous

mating system, a marker error rate of 0.01, a full likelihood method with medium-length runs,

and 3 simulations. Results of paternity and maternity were accepted with 95% confidence. I

conducted a sensitivity analysis to assess the consistency of results across different settings

(Appendix C3). I developed 9 sets of parentage analyses with different probabilities of actual

parents included in candidate males and females (0.5, 0.6, and 0.7 in males; 0.4, 0.5, and 0.6 in

females). The results of the sensitivity analysis showed consistent patterns across settings

(Appendix C3). I then set the probability of an actual male as 0.7 and female as 0.6 as a final model because the mean recapture rate during the spawning season was 0.69 and 0.59 in males and females, respectively (Appendix C4).

2.4 Statistical analyses

2.4.1 Variable Calculation

The four key variables to examine individual variation affecting reproductive ecology of bluehead chub were (1) mating system, (2) reproductive success of males and females, (3) variation in maximum nest size, and (4) female nest choice. Prior to the data preparation and calculation, I assessed PIT tag detection data to define individuals' actual participation in nests. I retained all female data regardless of the number of detections in each nest. However, male bluehead chub invest more time than females in securing the spawning pit on nests and moving pebbles (Wallin 1989; Sabaj et al. 2000). In addition, the absolute number of detections varied by each nest due to the variation in the number of tagged fish on nests. Thus, I used the relative detection of male on the nest to define actual participants. When equal to or less than 5 males were detected on a nest, I defined that males with > 0.1 relative detections actually participated in spawning. When more than 5 males were detected on a nest, I used a loose criterion (> 0.05) to define actual participants.

Response variables were calculated for three independent analyses (Table 3-1). First, reproductive success was defined as the number of YOY assigned to each male and female based on the result of parentage analysis. Second, nest size was calculated as the volume of elliptical cone (m^3). Male bluehead chub provide parental care by moving pebbles onto their nests after spawning (Wallin 1989; Sabaj et al. 2000). Thus, the maximum nest size was estimated by daily

recorded data. Third, I calculated the number of females on active nests using the antenna data. However, females could include an unknown number of immature males as well as females. Thus, I only accepted females that had reproductive success based on the result of parentage analysis.

I calculated 9 explanatory variables, which were included in different sets of analyses (i.e., reproductive success, maximum nest size, female nest choice) (Table 3-1). Body size (TL in mm) was either measured in the field or predicted for individuals which had been tagged before the spawning season and detected at PIT antennas without being recaptured in May and June. I used CMR data (688 individual) to estimate the body size of non-recaptured individuals during the spawning season using simple linear regression models (Appendix C5). The linear models were developed for each month. I conducted the CMR sampling twice in May; thus two data set were combined but I selected the larger body size when individuals were recaptured in both samplings. Finally, the estimated body size was determined when individuals actually appeared during the spawning (May or June).

The number of spawning was calculated by the number of nests where an individual was detected. The movement was estimated by the cumulative distance (20-m resolution) between nests where an individual was detected. The largest nest size was defined as maximum nest size where individuals were detected. Individuals displayed different proportions of detection on nests, while maximum nest size varied by the nest. Thus, I defined and calculated the "reproductive effort" as follow:

$$Reproductive\ effort_i = \frac{\left(\sum_{j=1}^{n} P_{i,j} \times S_j\right)}{n} \qquad (1)$$

where $P_{i,j}$ is the relative detection of individual i in nest j, S_j is the maximum nest size of nest j, and n is the number of nests where individual i participated. For instance, if a male participates

in the 'nest A' with the relative detection is 0.6 and the maximum nest size is 2 m^3, the effort of

the male on the nest A is 1.2. When this male participates in another nest and his reproductive

effort is 1.0, the total reproductive effort is 2.2 then the effort (see equation (1) above) is 1.1. The

size and number of males on each nest were estimated by identified individuals based on PIT

detection data. The active nest size was the average size of the nest when it was active. The

timing was estimated by the mean day of year fish that were detected at PIT antennas.

2.4.2 Mating system Analyses

A social network analysis (SNA) was used to visualize interactions among individuals

and mating system of bluehead chub. I created a two-dimensional matrix consisted of

associations between individuals (males and females) and nests based on PIT tag detection data.

I then constructed another matrix using the pedigree structure resulted from the parentage

analysis, and this matrix consisted of mating system with parental pair bonds, including sampled

and inferred males and females. An affiliation network was established for each matrix. The

number of mates by each individual was extracted from the result of the affiliation network of

mating system then compared with reproductive success (the number of YOY assigned by each

individual) using a linear model (LM). The SNA was fit using the *igraph* package (Csardi and

Nepusz 2006) in the R version 3.5.1 (R Development Core Team 2018).

2.4.3 Reproductive Success Analyses

A generalized linear model (GLM) was used to examine the relationship between

reproductive success and 6 explanatory variables (Table 3-1). Since the global model of the

Poisson GLM in the male analysis displayed overdispersion (residual deviance= 76 on 27 degree

of freedom), I used the Negative Binomial GLM to account for overdispersion. The Poisson GLM was used for the female analysis because I did not detect overdispersion in the global model (residual deviance= 13 on 38 degree of freedom). The male and female analyses included 6 explanatory variables (Table 3-1). In particular, the quadratic term was included as a predictor to account for a potentially unimodal response of reproductive success to male body size (Fig. 3-5a). The maximum nest size was analyzed using the LM, including 2 explanatory variables (Table 3-1). I examined female nest choice related to 3 explanatory variables using the Negative Binomial GLM to account for overdispersion (Poisson GLM, residual deviance= 64 on 14 degree of freedom). All explanatory variables were standardized by mean (mean = 0 and standard deviation = 3-1) to facilitate model convergence and allow direct comparison of effect sizes. The variance inflation factor (VIF) was used to assess multicollinearity between explanatory variables in each analysis. All pairwise VIF values were lower than 3; thus all explanatory variables were retained in global models (O'Brien 2007). A set of models was constructed using all possible combinations of variables. Models were compared using Akaike's information criterion corrected for small sample size (AIC_c). Competing models were identified when Akaike weights equal to or greater than 10% of the top-ranked model (Burnham and Anderson 2002: Appendix C8, C9, C10). The effect size was model-averaged across competing models, and the statistical significance was defined if 95% confidence interval (CI) did not overlap zero. Analyses were conducted in the R version 3.5.1 (R Development Core Team 2018).

3. Results

During the study period (April 1st – July 12th, 2017), mean daily water temperature in Shoal Creek was 18.7°C (range= 14.1 – 22.3), and water temperature increased gradually over

time. Precipitation occurred on 34 days, during which the mean daily value across these days was 9.9 mm (range= 0.3 – 57.7). In particular, the most severe rain events occurred during early the spawning season between April 17th and May 22nd. Water level was marginally influenced by precipitation (Spearman ρ= 0.33, *P*-value= 0.001: Appendix C6), but peak flows were strongly associated with rain events.

A total of 22 nests were located from April 15th to June 27th, while up to 70% of nests were active during a one-month period between May 10th and June 9th. I successfully monitored 18 nests but missed four nests, which were destroyed by high flows caused by severe rains, especially during the early spawning season. The mean active nest size was 0.017 m^3 (range= 0.009 – 0.027), and the mean maximum nest size was 0.047 m^3 (range= 0.015 – 0.116).

In total, 49 males and 277 potential females were detected at antennas but I considered 34 males as actual participants based on the relative detection. The result of parentage analysis showed that the paternity was assigned to 169 of 326 YOY (51.8% individuals) and maternity was assigned to 100 YOY (30.7%). A total of 16 males had reproductive success but one of these males was not detected in monitored nests, thus I only considered 15 males as successful spawners. In potential females, 45 of 277 individuals had reproductive success and I considered only these individuals as confirmed females. Reproductive success of male bluehead chub was highly skewed (mean: 10, range= 1 – 33), while females displayed less variability (mean= 2, range= 1 – 5). Mean body size of males was 138 mm (range= 103 – 180), and mean body size of females was 93 mm (range= 72 – 116). The distribution of body size exhibited bi- or tri-modal patterns in both sexes, perhaps indicating that both sexes may consist of multiples age classes (Appendix C7).

Male and female bluehead chub visited multiple nests during the spawning period (male, mean= 1.8, range= 1 – 5; female, mean= 4.0, range= 1 – 8: Fig. 3-3). Notably, females moved longer distances (mean= 112 m, range= 0 – 500) than males (mean= 14 m, range= 0 – 60). Bluehead chub mostly spawned between mid-May and mid-June (day of year: male, mean= 151, SD= 14; female, mean=160, SD= 10), which coincided with the peak spawning based on the number of located nests. Larger males tended to spawn earlier than smaller males (Spearman ρ= -0.41, P-value= 0.009, n= 34). Multiple males typically shared the same nest, but the number of males varied by nest (median= 4, range= 1 – 5: Fig. 3-3). The median of 11 females (range= 1 – 32) was detected per active nest (Fig. 3-3).

The result of the pedigree structure resulted from the parentage analysis inferred 11 unknown males and 54 unknown females (Fig. 3-4a), and these individuals produced offspring with sampled individuals. A total of 166 couples were reconstructed from the parentage analysis, and 43 of 166 couples were sampled individuals. The remaining social ties were 96 couples were pairs between sampled individuals and inferred individuals from the parentage analysis, and 27 couples were pairs between inferred individuals (Fig. 3-4a). Both sexes were more likely to mate with multiple mates (male, mean= 7.4, range= 1 – 26; female, mean= 1.7, range= 1 – 4: Fig. 3-4b), indicating that the mating system of bluehead chub was polygyandry. Reproductive success of both sexes was strongly associated with the number of partners (male, coefficient= 1.371, SE= 0.076, P-value< 0.001; female, coefficient= 0.898, SE= 0.131, P-value< 0.001: Fig. 3-4b).

The analyses identified 2 to 10 completing models in the male and female analyses and female nest choice analysis (Appendix C8,10). However, the top-ranked model of the maximum nest size model outperformed the other models (Appendix C9). Both linear and quadratic terms of body size was not an important factor for male reproductive success (linear, 95% CI= -9.161 –

0.572; quadratic, 95% CI= -5.188 – 3.119: Table 3-2 and Fig. 3-5a), which was supported by a manipulative field experiment examined the size-dependent reproductive success of male (Appendix C1). However, participating in larger nests (largest nest size: coefficient= 1.287, 95% CI= 0.345 – 0.902: Table 3-2 and Fig. 3-5c) with more investment (reproductive effort: coefficient= 0.544, 95% CI= 0.203 – 1.506: Fig. 3-5d) were significant factors that strongly increased male reproductive success. On the other hand, body size was the only significant factor that positively affected female reproductive success (coefficient= 0.802, 95% CI= 0.139 – 1.458: Table 3-2 and Fig. 3-5b).

The maximum nest size increased when multiple males shared the same nest (male number: coefficient= 0.699, 95% CI= 0.287 – 1.111: Table 3-3 and Fig. 3-6c), but did not depend on male size on nests (coefficient= -0.013, 95% CI= -0.429 – 0.395: Fig. 3-6a). Females preferred nests with multiple males (male number: coefficient= 0.616, 95% CI= 0.055 – 0.951: Table 3-4 and Fig. 3-6d), but they did not select nests based on male size (coefficient= -0.156, 95% CI= -0.515 – 0.179; Fig. 3-6b) and the active nest size.

4. Discussion

Larger nests and greater reproductive effort led to successful reproduction in males, and nest sharing (i.e., the number of males in nests) was an important behavioral trait that led to successful construction of large nest and attraction of females (Fig. 3-7). However, I found little evidence that male body size affected reproductive success and nest construction, while female body size was positively associated with reproductive success (Fig. 3-7). These findings suggest that male body size is not a primary factor for constructing larger nests, female choice, or reproductive success—a finding that is contrary to previous studies (Oliveira et al. 2000; Wong

et al. 2008; Serbezov et al. 2010; Peoples et al. 2011; Lehtonen et al. 2015). Perhaps male

cooperative behavior (i.e., nest sharing) serves to mediate the size-dependent reproductive

success (Taborsky 1994; Gross 1996; Sinervo and Zamudio 2001; Díaz-Muñoz et al. 2014).

Nest size, which affects male reproductive success in many fishes (Barber 2013), was an

important trait that led to successful reproduction in male bluehead chub. Notably, the quality of

nest (i.e., nest size) is a synergetic mechanism that enhances reproductive success by attracting

more females thus obtaining larger brood size, which stimulates males to invest more parental

care (Blais Lehtonen et al. 2007; Järvi-Laturi et al. 2008). In nest-building fishes, defending

territories and nests through aggressive behavior is a common reproductive strategy in males,

such as toadfish (Bose et al. 2018), stickleback (Barber et al. 2001), and sand goby (Lehtonen et

al. 2007). On the other hand, male bluehead chub were more likely to engage in sharing the same

nest (Wallin 1989), which is a typical form of male-male cooperation (Taborsky 2001; Díaz-

Muñoz et al. 2014). Multiple males sharing the same nest leads to larger nest size (Fig. 3-6c),

and allows increased reproductive success by providing more parental care (Kim et al. in

review), gaining access to more females (Fig. 3-6d), and attracting nest associates (Kim et al. in

review), which reduce predation risk through the dilution effect (Peoples and Frimpong 2016;

Silknetter et al. 2019).

In addition, male reproductive success was also associated with efforts on nests (i.e.,

reproductive effort). Male bluehead chub dig a small pit for spawning and move pebbles to

construct a nest as a form of parental care (Maurakis et al. 1991; Sabaj et al. 2000). When

multiple male bluehead chub shared the same nest, they were likely to compete with each other

by defending their positions on the nest, as similarly observed by Sabaj et al. (2000). This

finding may have valuable insights on complex relationships among cooperative males.

Although male bluehead chub engage in reproductive cooperation as co-breeders, such competitive behavior (i.e., defending their position) may cause reproductive skew even within a cooperative male group (Clutton-Brock 2002; Taborsky 2009). This may further explain why temporary spawners such as sneaker did not successfully reproduce and why other behavioral traits, such as the spawning frequency (the number of nests where males visited), did not increase reproductive success.

Nest sharing behavior had strong effects on maximum nest size. Nesting behavior of bluehead chubs is energetically costly because males move thousands of pebbles during and after spawning (Wallin 1989; 1992). In addition, suitable nesting sites are generally limited due to their habitat preference (Peoples et al. 2011). Male bluehead chub prefer shallower run habitats with a narrow range of water velocity and availability of nesting resources such as pebbles (Bolton et al. 2015; Peoples et al. 2016). Limited nesting sites and resources can act as strong environmental pressures on male bluehead chub to share the same nest, and this behavior can also be favored because it minimizes energy expenditure by providing parental care cooperatively (Taborsky 1994; Kim et al. in review). My findings further suggest that the unique nest sharing behavior which may have other types of male-male cooperation such as mutual tolerance and cooperative display (Díaz-Muñoz et al. 2014). Male bluehead chub displayed mutual tolerance when they shared the same nest but using their own spawning pit, and the strength of nest sharing (i.e., the number of males on nests) enabled them to attract more females as a form of cooperative display and coalition (Díaz-Muñoz et al. 2014).

I found little evidence that body size was an important factor for male reproductive success, nest construction, and female choice. This is surprisingly contrary to common findings that body size is positively correlated to male reproductive success in fishes (Basolo 2004;

Oliveira et al. 2000; Serbezov et al. 2010). Similarly, nest size is also positively associated with male body size in many nest-building fishes (Lindström 1992; Wong et al. 2008; Lehtonen et al. 2015; Bose et al. 2018). Kim et al. (in review) also observed that male body size plays a role in constructing larger nests in bluehead chub. A lack of effect of male body size could be explained by the size-dependent the timing of spawning of bluehead chub. Reproduction is strongly affected by environmental fluctuations (Oliveira et al. 1999; Warren et al. 2012), and the timing of spawning can lead to variation in reproductive success among individuals (Santucci et al. 2003; Suski and Ridgway 2007). Specifically, larger males spawned earlier than smaller males in Shoal Creek, similarly to findings in Kim et al. (in review). Meanwhile, frequent peak flows associated with heavy rains severely affected nesting activity of bluehead chub between April and mid-May. Severe flood events may have a negative impact on reproductive outcomes of early spawners (i.e., larger males), suggesting that external factors such as weather conditions can affect spawning activity regardless of individual traits, which may in turn affect the size-dependent reproductive success (Santucci et al. 2003; Warren et al. 2012).

Reproductive success of females was influenced solely by body size, and females exhibited a distinct preference through nest choice. Body size associated with reproductive success in females is common across many taxa (Honek 1993; Cox et al. 2003). These findings support the idea that internal characteristics (i.e., fecundity) are primary factors for reproductive success and overwhelm other individual traits including spawning frequency, movement, and nest quality (Wootton 1979; Trippel et al. 1997). Females assess multiple male traits, including body size, nest quality, parental care effort, for mate choice in fishes (Barber et al. 2001; Blais et al. 2004; Lehtonen et al. 2007; Bose et al. 2018). My findings typically showed that females favored a nest with multiple males; neither male body size nor nest size was sufficient to attract

females but the activeness (multiple males) of a nest was a determinant factor for female choice. This finding is likely to coincide with the preferences of nest associates on bluehead chub nests. Kim et al. (in review) reported a weak relationship between nest size and yellowfin shiner (nest associates) abundance, while yellowfin shiner prefer multiple males on nests. Additionally, reproductive outcomes of bluehead chub are strongly influenced by the presence or abundance of nest associates (Peoples and Frimpong 2016; Silknetter et al. 2019). Coupled with previous findings, these results have implications on nest sharing behavior as a key behavioral trait that attracts more nest associates and females and construct larger nests.

Bluehead chub were more likely to mate with multiple partners, particularly in males. Mating system is strongly affected by sexual selection (Andersson 1994; Bateman 1948), and having multiple partners is advantageous for increasing individual fitness through genetic diversity of offspring (Brooks and Couldridge 1999; Serbezov et al. 2010). In particular, reproductive success of male bluehead chub was highly skewed, and a relationship between reproductive success and the number of mates suggested that mating system was strongly influenced by sexual selection (Reynolds 1996). Various individual traits affect mating system, while commonly known trait is male body size, particularly in Salmonids (Garant et al. 2001; Serbezov et al. 2010). Specifically, larger males were more likely to occupy and spawn at better nests by mating with multiple females (Brooks and Couldridge 1999; Serbezov et al. 2010). However, the results of the male analysis showed that body size was not important for reproductive success of male bluehead chub, suggesting that other individual traits such as participating in larger nests and interacting with multiple males could be determinants of mating system. Since females were more likely to visit nests with multiple males, social interactions among males could be a key component of successful reproduction as well as forming a complex

mating system (Reynolds 1996), which is beyond the scope of this study but further investigations are warrants to reveal individual sociality affecting mating system and reproductive success.

This study monitored a single spawning season to characterize individual variation in reproductive success of bluehead chub. However, these findings may have valuable implications on the lifetime reproductive success of male bluehead chub. Larger males usually spawn earlier which is advantageous not only for occupying better territories and accessing more females (Noltie and Keenleyside 1987; Dupuis and Keenleyside 1988) but also for reducing predation risk of offspring through early emergence by allowing YOY to obtain a larger body size (Divino and Tonn 2007). To acquire such benefits, males would be faced with a reproductive trade-off between the current reproductive effort and future benefit associated with their body growth and survival over time (Gross 2005). However, my findings suggest that the body size effect would vary with environmental conditions. A severe drought affected the southeastern US (Williams et al. 2017) and provided stable flow conditions during the spawning season in 2016 (Kim and Kanno in press; Kim et al. in review). However, frequent peak flows occurred during the early spawning season in 2017. Such inter-annual environmental fluctuations could cause variation in the body size effect on nesting activity and explain why larger males were collected in Shoal Creek even though the body size was not an important trait for successful reproduction (Appendix C7). If there are stable flow conditions in spawning season, reproductive skew among males could be caused by body size rather than nest size or their nesting effort. Thus, future research is warranted to disentangle how the body size effect on reproductive success would vary by environmental conditions over individual's lifetime.

This study applied monitoring techniques using PIT tag antenna array to quantify individual variation and genetic parentage analysis to examine individual reproductive success. This integrated study, coupled with multiple techniques, successfully addressed individual variation in reproductive success of bluehead chub even they displayed aggregation on nests. However, this study has shortcomings. First, imperfect sampling should be considered, particularly in the natural stream. The CMR data and assignment rate of parentage analysis indicated that there was a possibility of imperfect detection of potential males and females. Additionally, several tagged adults did not attend nests during the spawning season. Since the study stream was an open system, fish could move out of the study reach. Similarly, the larval drift could affect the parentage assignment rate; most YOY collected from upstream were not assigned to candidate parents (*S. Kim* unpublished data). Thus, selecting closed systems as a study site or long-term stream blocking with depletion sampling may reduce such uncertainties. Second, the study design of nest monitoring in the natural system could not describe a detailed role of male bluehead chub, such as nest owner, co-breeder, satellite, and sneaker. I also observed social hierarchy among males, but the antenna data (the number of detections) was not sufficient to disentangle detailed behaviors and relationships between males. An application of visible marking techniques (e.g., visible implant elastomer) with video recording could reveal reproductive interactions between males, which are key components in male-male cooperation in behavioral ecology (Gross 1996; Díaz-Muñoz et al. 2014).

Reproduction is a key component in life history process that affects population dynamics and individual fitness (Brommer 2000). Understanding reproductive ecology of keystone species is important, especially when their individual traits have a crucial impact on biodiversity in freshwater ecosystems via reproductive mutualisms (Johnston 1994; Peoples et al. 2015). This

study illustrates mating system and individual variation in reproductive success of keystone species, bluehead chub. My findings have implications on how population and genetic structures would be affected by individual variation in reproductive success, and effects of individual variation may also have a strong impact on biodiversity in ecosystems due to positive reproductive interactions between bluehead chub and nest associates. In addition, the results of this study demonstrate that a commonly considered phenotypic trait (i.e., body size) does not always play an important role in nest-building species and the importance of this trait varies by sex. Further, male-male cooperation can mediate the size-dependent reproductive success by attracting more females and nest associates and resulting in larger nests via cooperative nest sharing behavior, which suggests a unique reproductive ecology in nest-building fishes.

Tables and Figures

Table 3-1. List and description of variables included in GLMs used to describe reproductive success of male and female bluehead chub.

Categories	Variables	Descriptions
Response Variables	Reproductive success[1]	The number of YOY assigned by each individual
	Maximum nest size[2]	Maximum nest size after spawning/construction
	Number of females[3]	The number of females involved the active nest
Phenotypic and behavioral traits	Body size (mm)[1]	The total length (mm) of individual
	Number of spawning[1]	The number of nests where individuals detected
	Movement (m)[1]	The cumulative distance (20-m resolution) between nests where individuals detected
	Reproductive effort[1]	The mean products of relative detection and maximum nest size
	Largest nest size (m^3)[1]	The maximum size of nest where individuals involved
	Timing[1]	The mean day of year when individuals were detected
	Male size (mm)[2,3]	The maximum size of male detected on the active nest
	Male number[2,3]	The number of males detected on the active nest
	Average nest size (m^3)[3]	The mean nest size while active

[1]Reproductive success analyses
[2]Nest construction analysis (i.e., maximum nest size)
[3]Female nest choice analysis

Table 3-2. Model-averaged parameter estimates, standard error (SE), and 95% confidence limits (CLs) to predict reproductive success of male and female bluehead chub. Significant explanatory variables are shown in bold.

Sex	Parameter	Estimate	SE	95 % CLs	
				Lower	Upper
Male	Intercept	0.462	0.277	-0.318	0.786
	Body size (linear)	-2.332	2.331	-9.161	0.572
	Body size (quadratic)	-0.440	1.837	-5.188	3.119
	Largest nest size	**1.287**	**0.345**	**0.902**	**2.741**
	Reproductive effort	**0.544**	**0.244**	**0.203**	**1.506**
	Number of spawning	-0.305	0.382	-1.184	0.291
	Movement	-0.408	0.344	-0.93	0.612
	Timing	-0.092	0.329	-1.101	0.273
Female	Intercept	0.315	0.310	-0.410	0.784
	Body size	**0.802**	**0.323**	**0.139**	**1.458**
	Largest nest size	0.061	0.152	-0.250	0.427
	Reproductive effort	-0.015	0.100	-0.235	0.190
	Number of spawning	0.019	0.134	-0.418	0.330
	Movement	0.035	0.099	-0.183	0.241
	Timing	0.255	0.107	-0.457	0.365

Table 3-3. Model-averaged parameter estimates, standard error (SE), 95% confidence limits (CLs), and odd ratio (OR) to predict the maximum nest size. Significant explanatory variables are shown in bold.

Parameter	Estimate	SE	95 % CLs	
			Lower	Upper
Intercept	0.000	0.182	-0.385	0.385
Male number	**0.699**	**0.193**	**0.287**	**1.111**
Male size	-0.013	0.196	-0.429	0.395

Table 3-4. Model-averaged parameter estimates, standard error (SE), 95% confidence limits (CLs), and odd ratio (OR) to predict female nest choice. Significant explanatory variables are shown in bold.

Parameter	Estimate	SE	95 % CLs	
			Lower	Upper
Intercept	**2.261**	**0.164**	**1.942**	**2.554**
Male number	**0.616**	**0.194**	**0.055**	**0.951**
Male size	-0.156	0.178	-0.515	0.179
Active nest size	0.373	0.254	-0.223	0.671
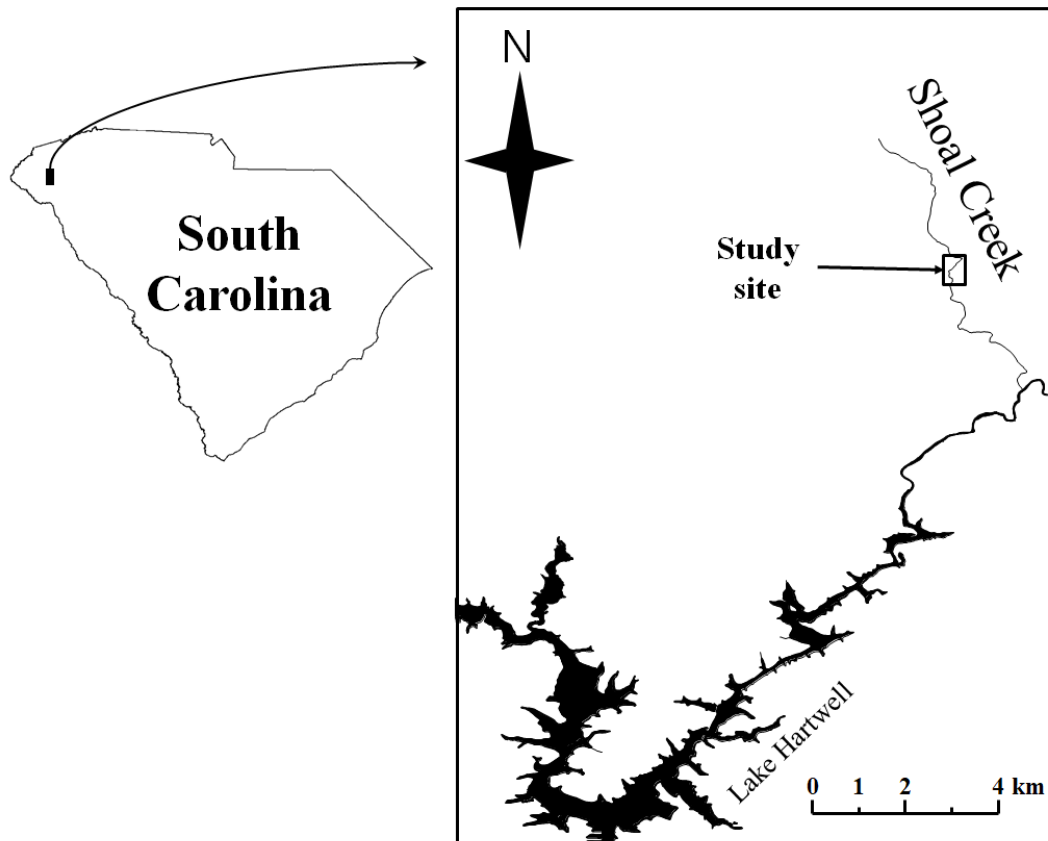
Fig. 3-1. Map of study stream in the Savannah River Basin in South Carolina, USA. Waterways (lakes and streams) are shown in black. The black rectangle indicates the study area in Shoal Creek.
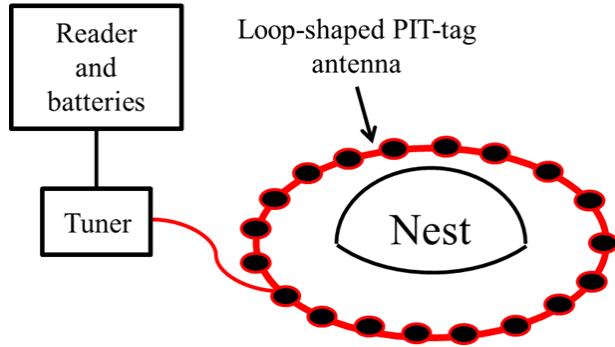
(a)   **(b)**

Fig. 3-2. (a) A conceptual design of the loop-shaped PIT antenna. PIT antenna surrounded a nest and the antenna was connected to tuner and reader to record the detection of unique individuals. (b) Photo showing the installation of PIT antenna while the nest was active.
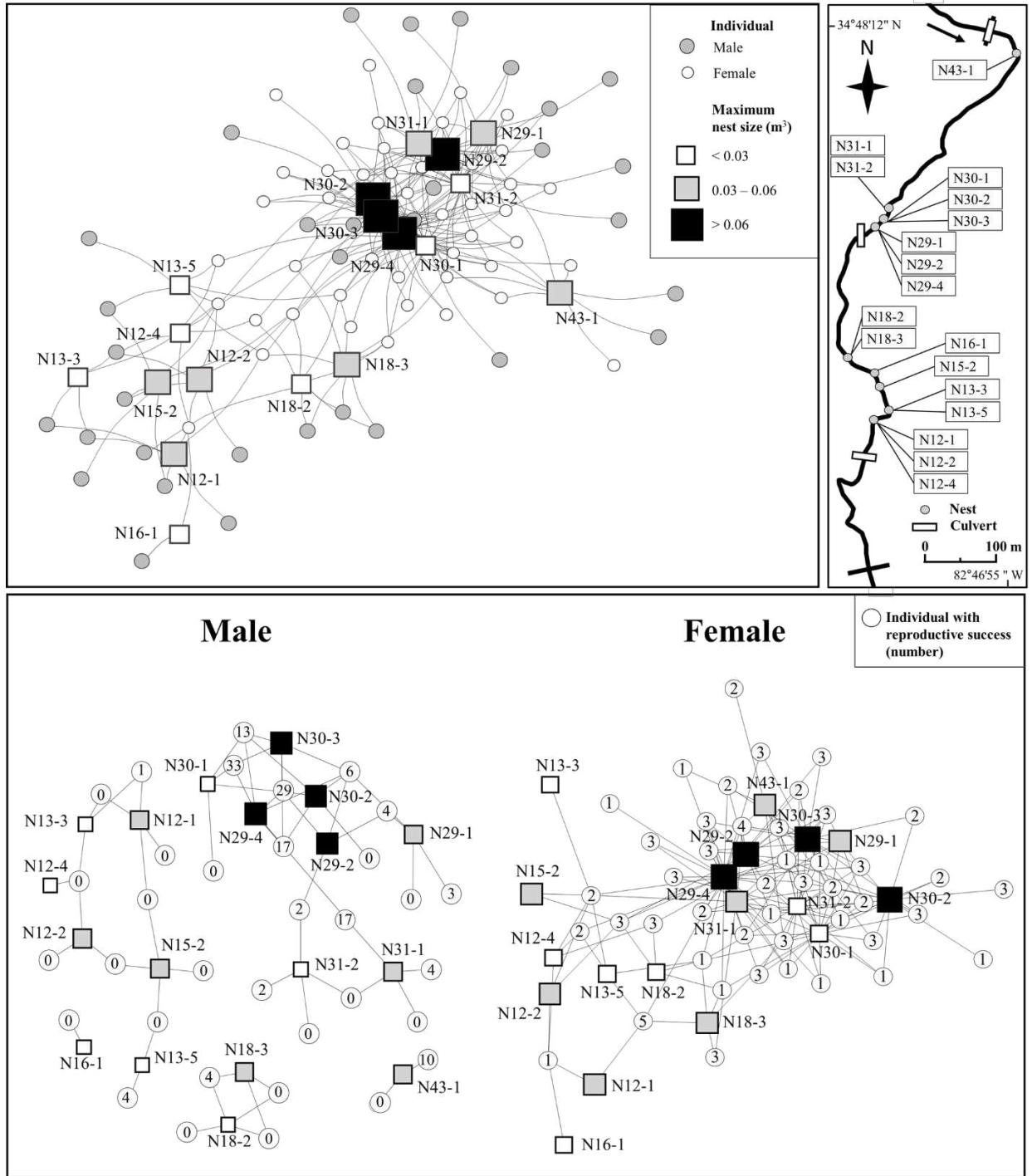
Fig. 3-3. Affiliation network showing spawning pattern of male and female bluehead chub based on PIT antenna data. Social ties (line) indicate nests where individuals visited during the spawning season.
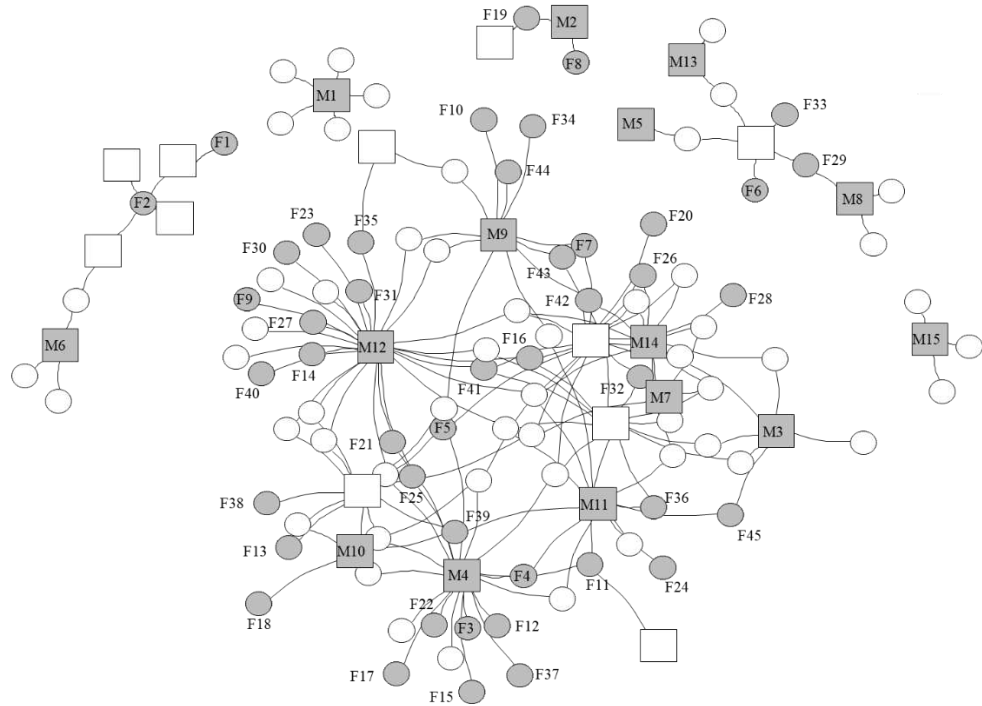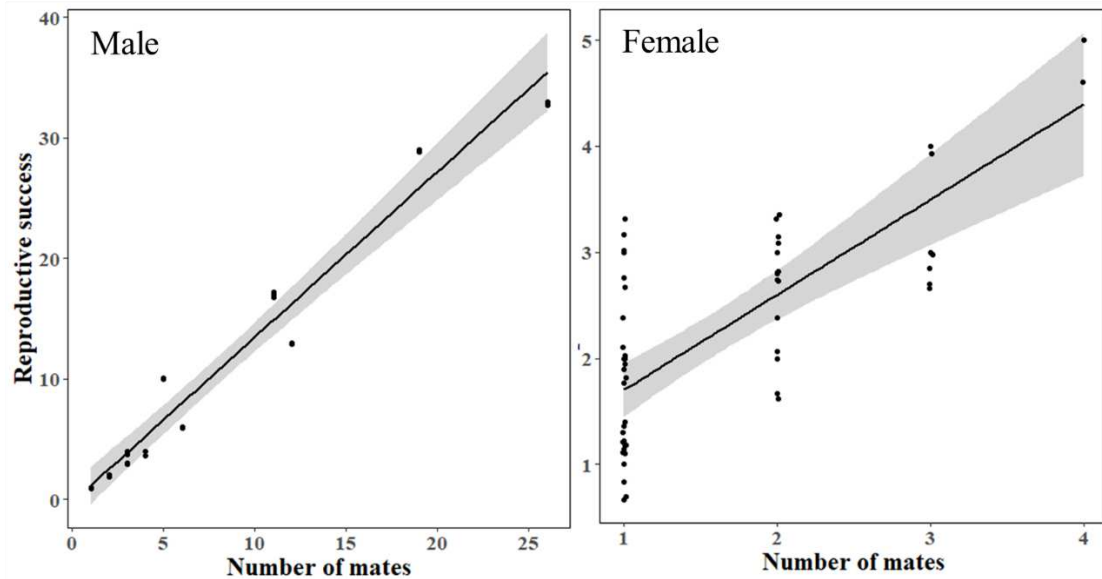
Fig. 3-4. Affiliation network showing (a) mating system of bluehead chub based on the result of parentage analysis. Social ties (lines) indicate the mating pattern between males (squares) and females (circles). The gray color indicates sampled and genotyped individuals and the white color is inferred individual by the parentage analysis; and (b) relationships between the number of mates and reproductive success of male (n=15) and female (n=34). Black lines indicate mean response and gray shading show 95% confidence interval.
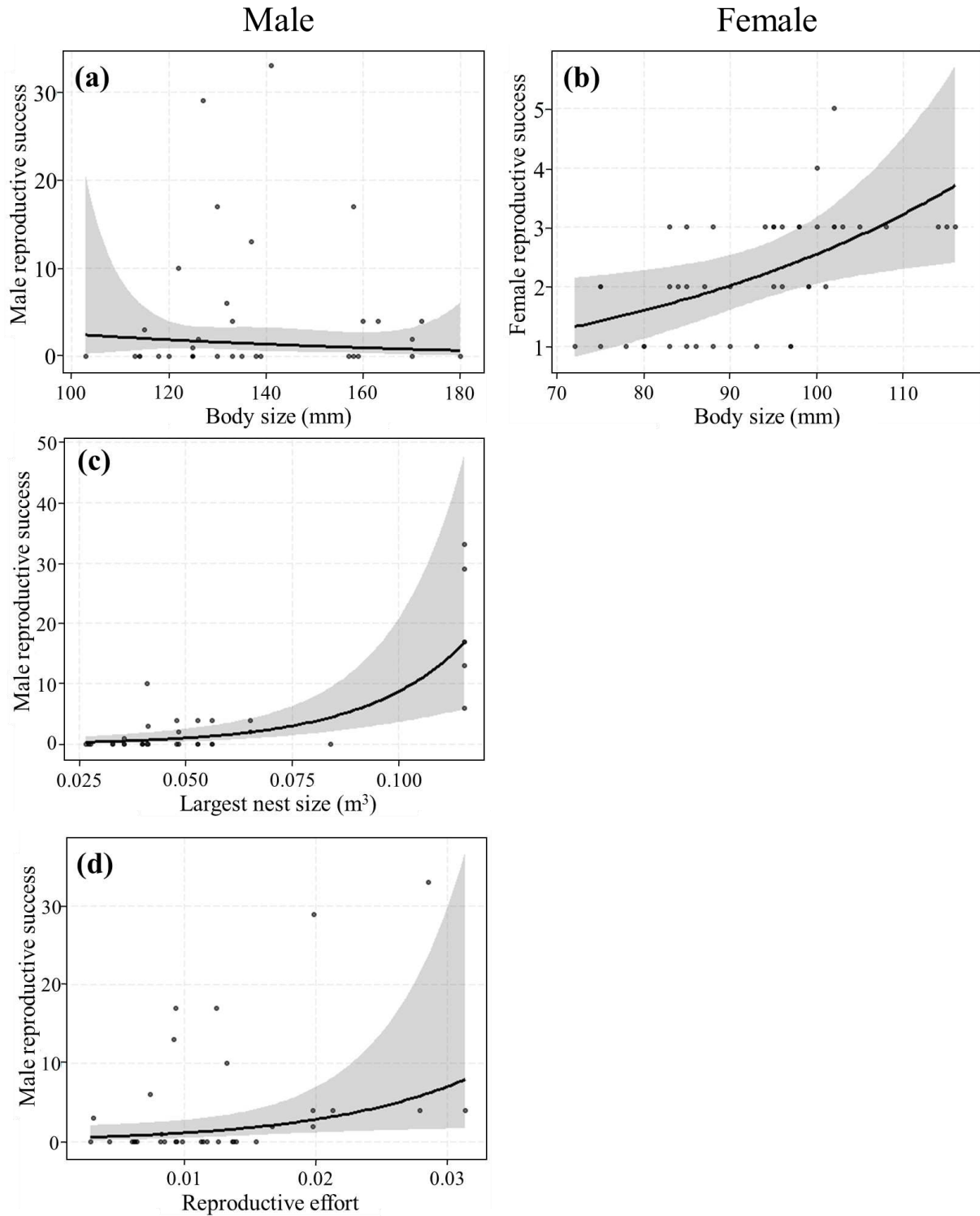
Fig. 3-5. Model-predicted effects of (a) body size, (c) largest nest size, and (d) reproductive effort on reproductive success of male bluehead chub (n= 34); and (b) body size on female reproductive success (n= 45). Black lines indicate mean response and gray shading show 95% confidence interval.
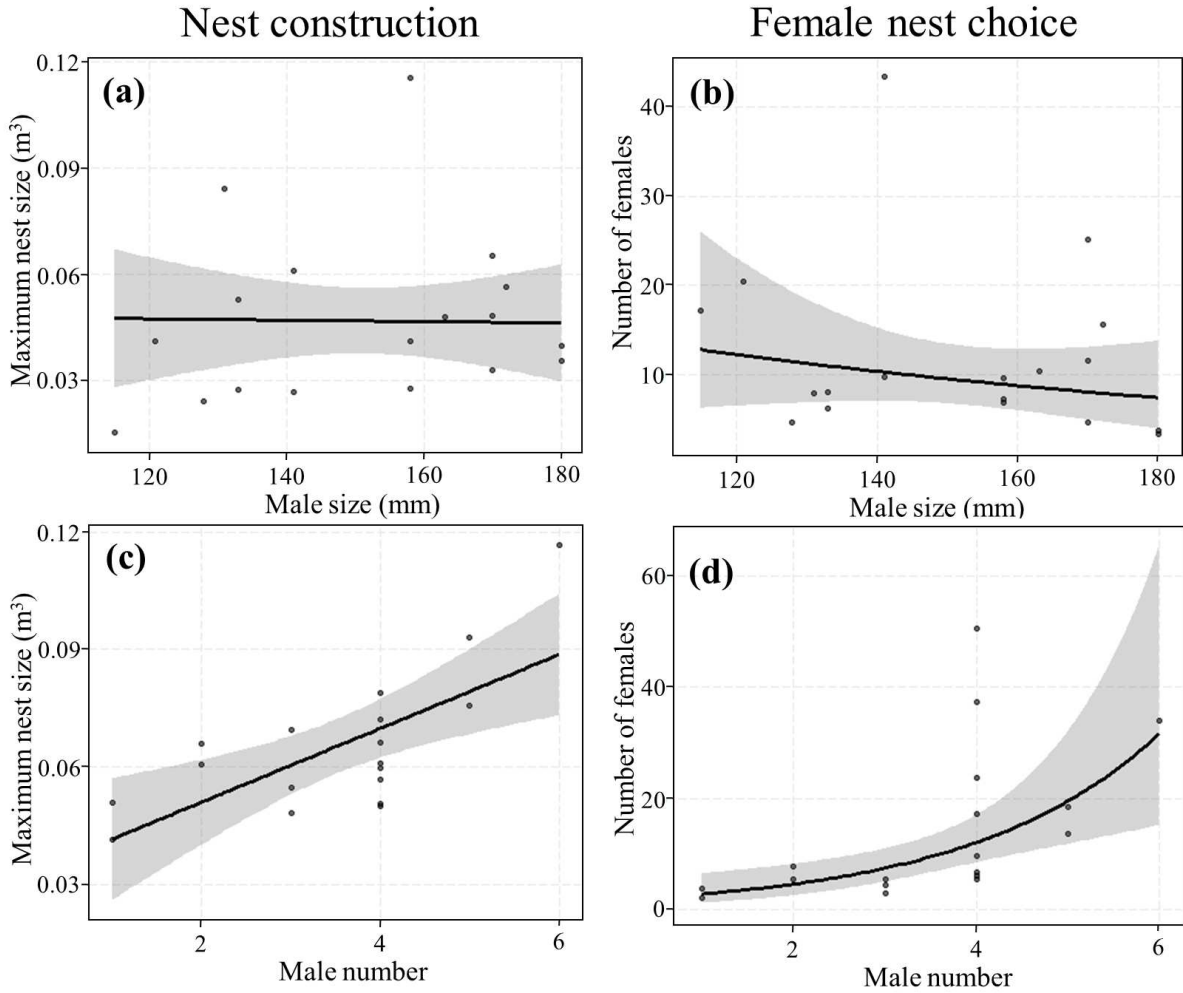
Fig. 3-6. Model-predicted effects of (a) male body size and (c) the number of males on maximum nest size (n= 18); and (b) male size and (d) the number of males on female nest choice (n= 18). Black lines indicate mean response and gray shading show 95% confidence interval.
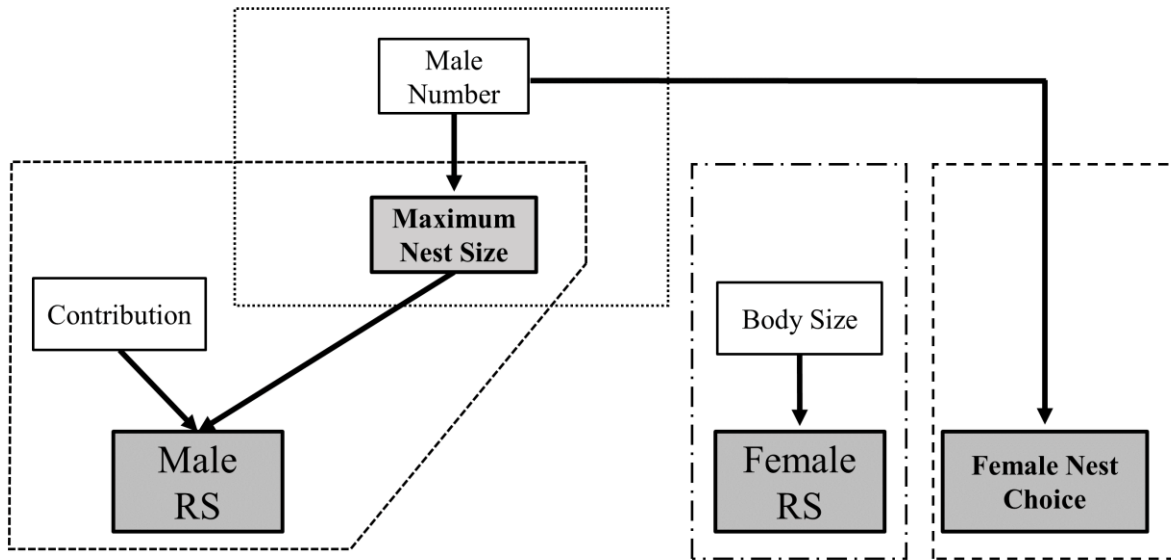
Fig. 3-7. A diagram represents the synthesis of results. Different shape of dotted polygons indicates 4 different sets of analyses such as the analysis of male and female reproductive success, maximum nest size, and female mate choice. Gray boxes represent response variables in each analysis, and white boxes with arrows indicate significant explanatory variables.

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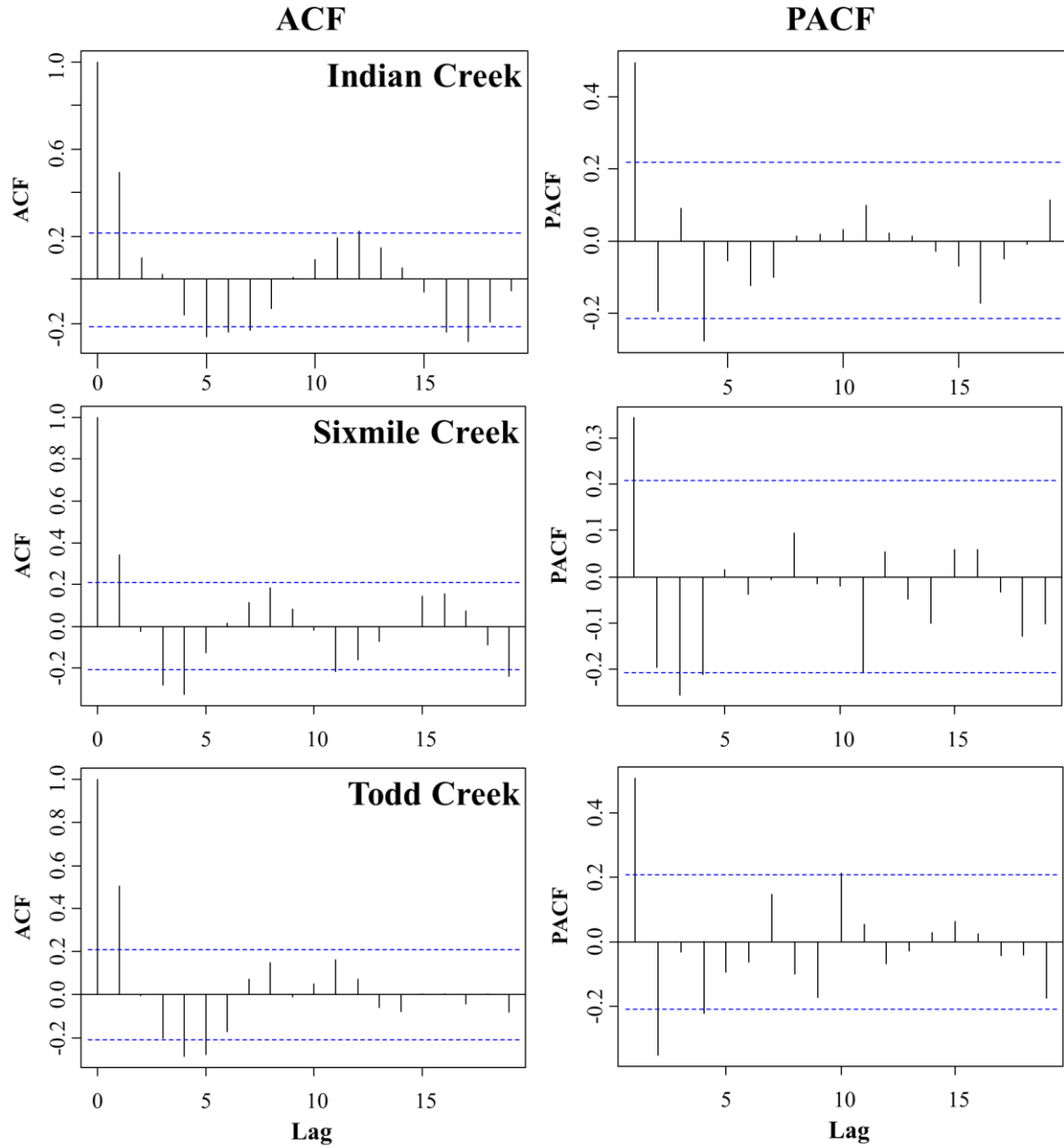
Appendix A1. Auto-correlation function (ACF) and partial auto-correlation function (PACF) plots from selected GLMs in each stream. Blue dashed lines indicate the statistical significance was set at $\alpha < 0.05$.

Appendix A2. List of candidate generalized linear auto-regressive and moving average models by ascending values of Akaike's information criterion corrected for small sample size (AIC_c). Models differed in the combination of polynomial auto-regressive (AR) and moving average (MA) terms. Significant AR and MA terms ($\alpha < 0.05$) are shown in bold.

Stream	Model[1]	AR	MA	Wald test (*P*-value)	LR test (*P*-value)	ACF Residual[2]	AIC_c	ΔAIC_c	Weight
Indian Creek	1	**1, 16**		< 0.001	< 0.001	N	142.66	0.00	0.60
	2	**1, 16**	6	< 0.001	< 0.001	N	143.59	0.93	0.38
	3	**1**	6	< 0.001	< 0.001	N	151.10	8.44	0.01
	4	1		< 0.001	< 0.001	N	151.21	8.55	0.01
	5	**16**	6	< 0.001	0.002	Y	161.17	18.51	0.00
	6	**16**		0.003	0.002	Y	166.52	23.86	0.00
	7		6	0.005	0.031	Y	167.47	24.81	0.00
Sixmile Creek	1	**3, 6, 11**	1	< 0.001	< 0.001	N	88.27	0.00	0.25
	2	**3, 11**	1	< 0.001	0.001	N	89.05	0.78	0.17
	3	**3, 6**	1	< 0.001	< 0.001	N	89.47	1.20	0.14
	4	**11**	1	< 0.001	< 0.001	Y	89.48	1.21	0.14
	5	**6, 11**	1	< 0.001	< 0.001	Y	89.62	1.35	0.13
	6	**3**	1	< 0.001	< 0.001	Y	89.91	1.64	0.11
	7	-	1	< 0.001	< 0.001	Y	92.38	4.11	0.03
	8	6	1	0.003	0.002	N	94.48	6.21	0.01
	9	**3, 6, 11**	-	0.006	< 0.001	Y	95.77	7.50	0.01
	10	**11**	-	0.006	< 0.001	Y	95.93	7.66	0.01
	11	**3, 6**	-	0.007	< 0.001	Y	96.00	7.73	0.01
	12	**6, 11**	-	0.011	< 0.001	Y	96.81	8.54	0.00
	13	**3**	-	0.014	0.005	Y	97.45	9.18	0.00
	14	**3, 11**	-	0.015	< 0.001	Y	98.41	10.14	0.00
	15	6	-	-	-	-	-	-	-
Todd Creek	1	**2, 4, 6, 15**	1	< 0.001	< 0.001	N	152.56	0.00	0.63
	2	**2, 4, 6**	1	< 0.001	< 0.001	Y	155.07	2.51	0.18
	3	**4, 6, 15**	1	< 0.001	< 0.001	Y	155.29	2.73	0.16
	4	**4, 6**	1	< 0.001	< 0.001	Y	159.27	6.71	0.02
	5	2, **4**	1	< 0.001	< 0.001	N	172.67	20.11	0.00
	6	**4**	1	< 0.001	< 0.001	N	172.79	20.23	0.00
	7	**4**, 15	1	< 0.001	< 0.001	N	173.93	21.37	0.00
	8	2, **4**, 15	1	< 0.001	< 0.001	N	175.13	22.57	0.00
	9	15	1	< 0.001	< 0.001	N	187.88	35.32	0.00
	10	2	1	-	-	-	-	-	-
	11	6	1	-	-	-	-	-	-
	12	2, 6	1	-	-	-	-	-	-
	13	2, 15	1	-	-	-	-	-	-
	14	6, 15	1	-	-	-	-	-	-
	15	2, 6, 15	1	-	-	-	-	-	-

[1]Blank models indicated convergence failures in the Fisher Scoring iteration method.
[2]Auto-correlation function (ACF) of Pearson residual plots was visually assessed. Y: significant residuals detected in auto-correlation; N: no significant residual.
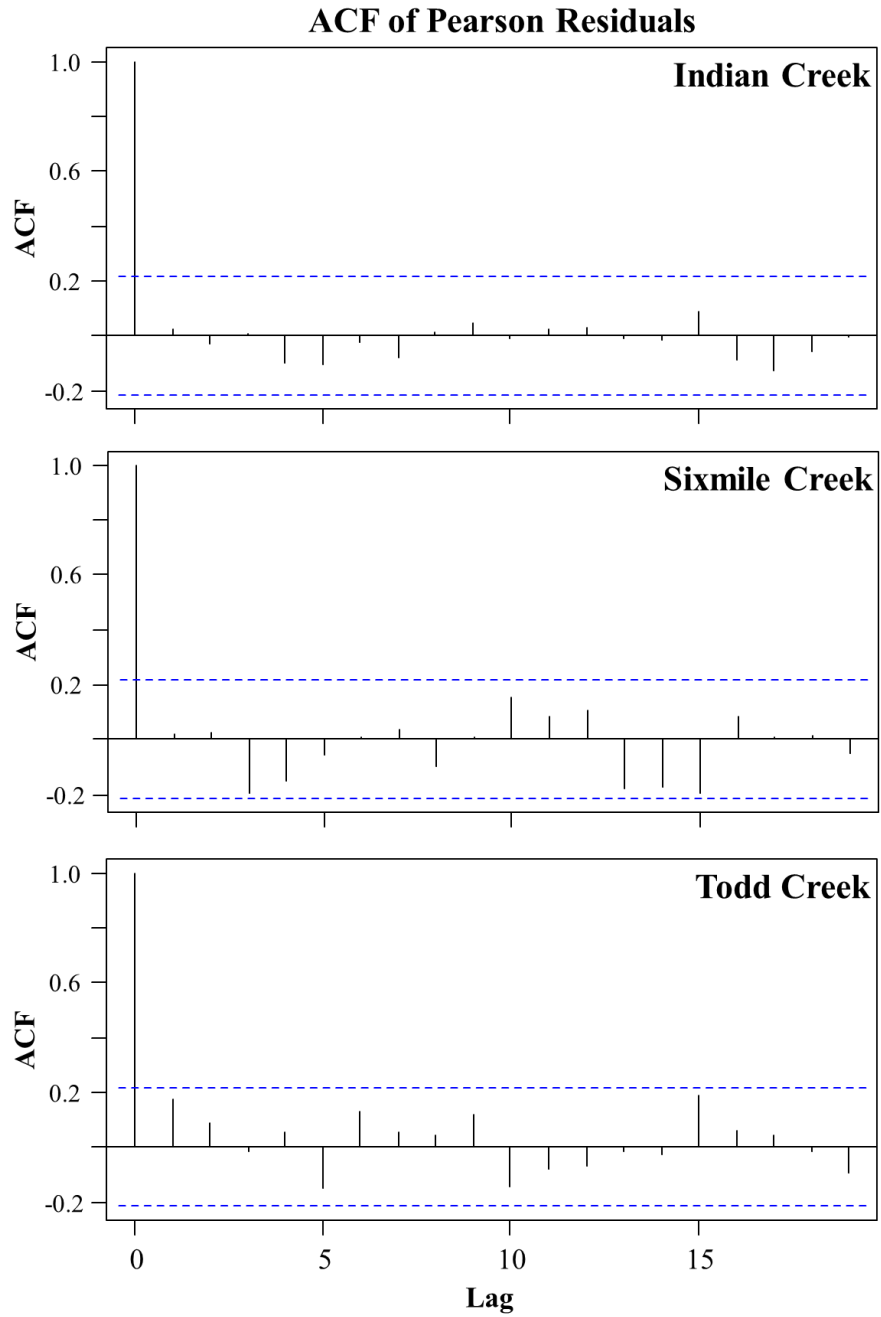
Appendix A3. Auto-correlation function (ACF) of Pearson residual plots from the generalized linear auto-regressive moving average model in each study stream. Dotted blue lines indicate 95 % confidence intervals.

Appendix B1. Results of six path models depicting abiotic and biotic variables affecting nest size and utilization by yellowfin shiner. The diagram of global model is shown in Fig. 2. Paths show hypothesized causality with effect size and significance. The significant paths (*P*-value < 0.05) are shown in bold. Hypothesized causalities or variables were removed based on their significance. Each model was evaluated based on the global goodness of fit (χ^2 test, $\alpha > 0.05$), root mean square error of approximation (RMSEA < 0.05 acceptable), comparative fit index (CFI > 0.9 acceptable), and the number of pathways (N ≤ 14) based on collected sample size. The final model (Model 6) was chosen until all variables were significant.

Model	Path	Estimate	S.E.	Z-value	*P*-value
	Photoperiod → Water temperature	**0.760**	**0.079**	**9.622**	< 0.001
	Photoperiod → Nest type	**0.240**	**0.045**	**5.366**	< 0.001
	Precipitation → Water temperature	-0.128	0.081	-1.571	0.116
	Precipitation → Water level	0.051	0.102	0.499	0.618
	Water level → Velocity	**0.469**	**0.103**	**4.553**	< 0.001
	Water level → Depth	0.168	0.117	1.435	0.151
	Water temperature → Male size	**-0.390**	**0.118**	**-3.313**	0.001
	Velocity → Male size	-0.115	0.11	-1.042	0.298
	Depth → Male size	0.083	0.093	0.890	0.374
	Male size → Nest type	0.105	0.068	1.542	0.123
Model1	**Male size → Nest building**	**0.383**	**0.129**	**2.968**	0.003
(global model)	Male size → Nest duration	0.252	0.131	1.926	0.054
χ^2 test: *p* < 0.001	Male size → Active nest size	0.203	0.104	1.959	0.050
CFI = 0.56	**Male size → Male number**	**0.397**	**0.11**	**3.620**	< 0.001
RMSEA = 0.18	Male number → Nest building	0.052	0.104	0.498	0.619
N = 26	Male number → Nest type	0.059	0.063	0.937	0.349
	Male number → Nest duration	0.101	0.106	0.959	0.338
	Male number → Active nest size	**0.443**	**0.186**	**2.386**	0.017
	Nest type → Maximum nest size	**0.477**	**0.171**	**2.796**	0.005
	Nest building → Maximum nest size	**0.216**	**0.079**	**2.717**	0.007
	Male size → Maximum nest size	**0.200**	**0.098**	**2.051**	0.040
	Male number → Maximum nest size	**0.509**	**0.129**	**3.953**	< 0.001
	Nest duration → Number of shiner	**0.308**	**0.088**	**3.508**	< 0.001
	Active nest size → Number of shiner	0.162	0.169	0.959	0.338
	Male size → Number of shiner	0.181	0.098	1.843	0.065
	Male number → Number of shiner	**0.318**	**0.115**	**2.768**	0.006
	Photoperiod → Water temperature	**0.745**	**0.080**	**9.324**	< 0.001
	Photoperiod → Nest type	**0.239**	**0.045**	**5.356**	< 0.001
	Water temperature → Male size	**-0.367**	**0.107**	**-3.442**	0.001
Model2	Male size → Nest type	0.102	0.069	1.486	0.137
(precipitation, water	**Male size → Nest building**	**0.393**	**0.129**	**3.043**	0.002
level, velocity, and depth	**Male size → Nest duration**	**0.258**	**0.13**	**1.989**	0.047
were removed from	Male size → Active nest size	0.201	0.104	1.933	0.053
Model1)	**Male size → Male number**	**0.398**	**0.109**	**3.639**	< 0.001
χ^2 test: *p* < 0.001	Male number → Nest building	0.048	0.103	0.464	0.643
CFI = 0.72	Male number → Nest type	0.06	0.063	0.954	0.340
RMSEA = 0.18	Male number → Nest duration	0.098	0.105	0.936	0.349
N = 20	**Male number → Active nest size**	**0.444**	**0.186**	**2.390**	0.017
	Nest type → Maximum nest size	**0.479**	**0.170**	**2.810**	0.005
	Male number → Nest building	0.048	0.103	0.464	0.643

(*Continues*)

	Nest building → Maximum nest size	**0.213**	**0.079**	**2.708**	**0.007**
	Male size → Maximum nest size	**0.204**	**0.096**	**2.126**	**0.034**
	Male number → Maximum nest size	**0.508**	**0.129**	**3.953**	**< 0.001**
Model2	**Nest duration → Number of shiner**	**0.308**	**0.088**	**3.494**	**< 0.001**
	Active nest size → Number of shiner	0.164	0.169	0.971	0.331
	Male size → Number of shiner	0.180	0.099	1.814	0.070
	Male number → Number of shiner	**0.317**	**0.115**	**2.765**	**0.006**
	Photoperiod → Water temperature	**0.745**	**0.080**	**9.324**	**< 0.001**
	Photoperiod → Nest type	**0.239**	**0.045**	**5.340**	**< 0.001**
	Water temperature → Male size	**-0.369**	**0.106**	**-3.499**	**< 0.001**
	Male size → Nest type	0.101	0.067	1.502	0.133
	Male size → Nest building	**0.392**	**0.129**	**3.041**	**0.002**
Model 3	**Male size → Nest duration**	**0.258**	**0.130**	**1.987**	**0.047**
(Active nest size was	**Male size → Male number**	**0.399**	**0.110**	**3.635**	**< 0.001**
removed form Model2)	Male number → Nest building	0.047	0.103	0.458	0.647
χ^2 test: $p = 0.62$	Male number → Nest type	0.056	0.061	0.917	0.359
CFI = 0.99	Male number → Nest duration	0.102	0.105	0.967	0.333
RMSEA < 0.001	**Nest type → Maximum nest size**	**0.453**	**0.163**	**2.780**	**0.005**
N = 17	**Nest building → Maximum nest size**	**0.222**	**0.079**	**2.804**	**0.005**
	Male size → Maximum nest size	**0.199**	**0.098**	**2.039**	**0.041**
	Male number → Maximum nest size	**0.502**	**0.128**	**3.930**	**< 0.001**
	Nest duration → Number of shiner	**0.296**	**0.091**	**3.260**	**0.001**
	Male size → Number of shiner	**0.217**	**0.097**	**2.235**	**0.025**
	Male number → Number of shiner	**0.397**	**0.087**	**4.591**	**0.000**
	Photoperiod → Water temperature	0.745	0.080	9.324	< 0.001
	Photoperiod → Nest type	0.239	0.045	5.344	< 0.001
	Water temperature → Male size	-0.368	0.106	-3.473	0.001
	Male size → Nest type	0.101	0.067	1.498	0.134
Model 4	**Male size → Nest building**	0.412	0.118	3.482	< 0.001
(hypothesized causality	**Male size → Nest duration**	0.258	0.130	1.989	0.047
between Male number and	**Male size → Male number**	0.400	0.110	3.647	< 0.001
Nest building was	Male number → Nest type	0.056	0.061	0.917	0.359
removed form Model3)	Male number → Nest duration	0.101	0.105	0.967	0.334
χ^2 test: $p = 0.65$	**Nest type → Maximum nest size**	0.453	0.163	2.783	0.005
CFI = 0.99	**Nest building → Maximum nest size**	0.223	0.080	2.804	0.005
RMSEA < 0.001	**Male size → Maximum nest size**	0.199	0.097	2.047	0.041
N = 16	**Male number → Maximum nest size**	0.502	0.127	3.942	< 0.001
	Nest duration → Number of shiner	0.296	0.091	3.264	0.001
	Male size → Number of shiner	0.216	0.097	2.229	0.026
	Male number → Number of shiner	0.398	0.086	4.605	< 0.001
Model 5	**Photoperiod → Water temperature**	**0.745**	**0.080**	**9.324**	**< 0.001**
(hypothesized causality	**Photoperiod → Nest type**	**0.239**	**0.045**	**5.322**	**< 0.001**
between Male Number	**Water temperature → Male size**	**-0.368**	**0.106**	**-3.464**	**0.001**
and Nest type was	**Male size → Nest type**	**0.125**	**0.058**	**2.153**	**0.031**
removed from Model4)	**Male size → Nest building**	**0.412**	**0.118**	**3.478**	**0.001**
N = 15, χ^2 test: $p = 0.66$,	**Male size → Nest duration**	**0.255**	**0.130**	**1.953**	**0.041**
CFI = 0.99, RMSEA <	**Male size → Male number**	**0.403**	**0.110**	**3.654**	**< 0.001**
0.001	Male number → Nest duration	0.106	0.105	1.004	0.315

(*Continues*)

Continued

	Nest type → Maximum nest size	**0.460**	**0.162**	**2.832**	0.005
	Nest building → Maximum nest size	**0.223**	**0.079**	**2.815**	0.005
	Male size → Maximum nest size	**0.199**	**0.097**	**2.054**	0.040
Model 5	Male number → Maximum nest size	**0.501**	**0.126**	**3.964**	< 0.001
	Nest duration → Number of shiner	**0.296**	**0.091**	**3.257**	0.001
	Male size → Number of shiner	**0.214**	**0.097**	**2.206**	0.027
	Male number → Number of shiner	**0.400**	**0.086**	**4.654**	< 0.001
Model 6 (final model: hypothesized causality between Male Number and Nest type was removed from Model5) N = 14 χ^2 test: $p = 0.66$ CFI = 0.99 RMSEA < 0.001	Photoperiod → Water temperature	**0.745**	**0.080**	**9.324**	< 0.001
	Photoperiod → Nest type	**0.239**	**0.045**	**5.319**	< 0.001
	Water temperature → Male size	**-0.367**	**0.106**	**-3.452**	0.001
	Male size → Nest type	**0.124**	**0.058**	**2.132**	0.033
	Male size → Nest building	**0.412**	**0.118**	**3.479**	0.001
	Male size → Nest duration	**0.299**	**0.122**	**2.449**	0.014
	Male size → Male number	**0.404**	**0.11**	**3.659**	< 0.001
	Nest type → Maximum nest size	**0.457**	**0.162**	**2.824**	0.005
	Nest building → Maximum nest size	**0.223**	**0.079**	**2.815**	0.005
	Male size → Maximum nest size	**0.201**	**0.096**	**2.083**	0.037
	Male number → Maximum nest size	**0.499**	**0.126**	**3.955**	< 0.001
	Nest duration → Number of shiner	**0.298**	**0.091**	**3.270**	0.001
	Male size → Number of shiner	**0.212**	**0.097**	**2.180**	0.029
	Male number → Number of shiner	**0.402**	**0.086**	**4.682**	< 0.001

Appendix C1. Field experiment to examine the size-dependent reproductive success of male bluehead chub

1. Field methods

To examine the size-dependent reproductive success in male bluehead chub, I constructed experiment units (EUs) in Sixmile Creek (34°49'22" N, 82°49'39"W) in May 2017. Sixmile Creek was selected based on the habitat similarity to Shoal Creek, and stream reach was characterized by a series of riffle-pool sequence covered by riparian trees. The stream reach was separated into 12 EUs by placing mesh block nets (mesh size: 3.2 mm) across the stream bed (Fig. C1-1a). The mesh block nets were supported by steel posts and two-panel strips of 5×10 cm welded fencing (Peoples and Frimpong, 2016; Silknetter et al. 2019). The height of block nets was 122 cm, with 30 cm above the ordinary high-water mark. To prevent fish movement between EUs, block nets were anchored by 23 kg sandbags. Each EU contained at least one riffle-pool sequence to provide habitats for spawning and feeding (Peoples et al. 2011; Bolton et al. 2015). Two sets of experiments (each set had 12 EUs) were established between May 7th – 12th and 27th – 31st.

Experimental units were cleared of fish using an eight-pass electrofishing removal method or until no fish were captured (Fig. C1-1b). After the fish removal, I collected bluehead chub in Sixmile Creek and other nearby streams within 10 km of the study site. A pair of male bluehead chub of different sizes (large versus small males), 15 females (70 mm or greater in total length with visibly engorged abdomens; Jenkins and Burkhead 1994) were stocked in each EU. Females without engorged abdomens were excluded to reduce the potential for mistakenly stocking immature males. The mean body size of larger and smaller male was 174.2 mm (range = 145 – 205) and 130.0 mm (range = 102 – 160), respectively. The mean body size of females

was mean= 94 mm (range= 70 – 136). Anal fins were collected from all stocked fish for genetic analysis.

Once EUs were established, daily observations were conducted to determine spawning activity. Eggs were collected three days after nest construction had initially been observed to provide the time for spawning and avoid the risk of eggs hatching into larvae (Peoples and Frimpong, 2016; Silknetter et al. 2019). To collect eggs from nests, I placed a fine-mesh drift net (500-µm mesh size) immediately downstream of the nest. Pebbles were removed from the nest by hand allowing eggs to drift into the net. Once the nest had been completely deconstructed, eggs were preserved in 99% ethanol.

2. Genetic Analyses

I used a set of 8 microsatellite loci (Cushman et al. in review) to conduct genetic parentage analysis. Genetic analyses were conducted at the Hollings Marine Laboratory in Charleston, South Carolina. Laboratory protocols are detailed in Cushman et al. (in press). I used the software CERVUS 3.07 (Kalinowski et al. 2007) for parentage analysis in the field experiment sample. The settings used in CERVUS were as follows: the number of simulated offspring was 5000, proportion of parents sampled was 100%, and a genotype error rate was 0.01. The result of paternity was accepted with 95% confidence. In some EUs, I subsampled eggs due to higher egg abundance. When 35 or fewer eggs were collected from an EU, all eggs were identified and parentage analysis was conducted to assign the large or small males; reproductive success (number of eggs) was determined. For EUs with > 35 eggs, I subsampled eggs and then identified species and paternity. The proportion of paternity was used to infer reproductive success of large versus small males.

3. Statistical analyses

An analysis of covariance (ANCOVA) was used to examine the size-dependent reproductive success of male bluehead chub in six EUs. The response variable was the proportion of eggs assigned by each male. I included two size classes (i.e., large vs. small males) as a group effect and EU as a block effect. Difference in total length of paired males in each EU was included as a continuous in case the relative difference in male body size may affect reproductive outcomes. The interaction between size class and body size was included in the ANCOVA. All statistical analyses were conducted in the R version 3.5.1 (R Development Core Team 2018), and statistical significance was set at $\alpha < 0.05$.

4. Results

Bluehead chub constructed nests and successfully spawned in 6 out of 24 EUs. Based on EUs with successful spawning, the mean body size of larger and smaller male was 157 mm (range= 145 – 185) and 122 mm (range= 111 – 136), respectively (Fig. S2). A total of 139 eggs were collected across EUs. In two EUs, only the smaller male successfully reproduced, while the larger male had reproductive success in two other EUs (Fig. C1-2). Both males reproduced together in two EUs, but the proportion of reproductive success differed by size class. The result of ANCOVA showed that neither the size class ($F_{(1, 0.017)}$= 0.061, P-value= 0.81) nor body size ($F_{(1, 0.055)}$= 0.020, P-value= 0.69) including their interaction term ($F_{(1, 0.024)}$= 0.086, P-value= 0.78) had an effect on reproductive success (Table C1-1), suggesting the body size was not a primary factor for male fitness.

Table C1-1. Summary of the analysis of covariance for reproductive success of male bluehead chub by group effect (size class) and measured body size (mm) in experiment units.

Effect	Sum of squares	df	Mean square	F	P-value
Body size	0.0551	1	0.0551	0.0196	0.668
Group	0.0169	1	0.0168	0.0605	0.812
Body size: Group	0.0241	1	0.0241	0.0864	0.776
Error	2.2303	8	0.0279		
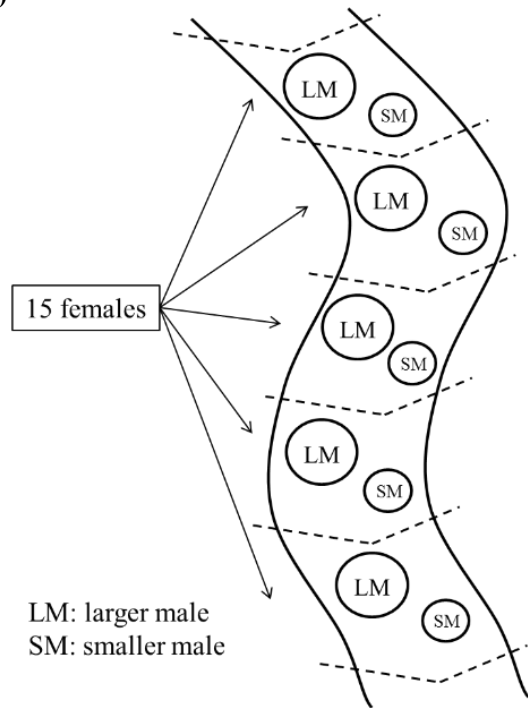
Fig. C1-1. (a) A conceptual diagram of experimental units. Two males of different size and 15 females were stocked after fish had been removed in each experimental unit. (b) Double backpack electrofishing to remove fish in experimental units.
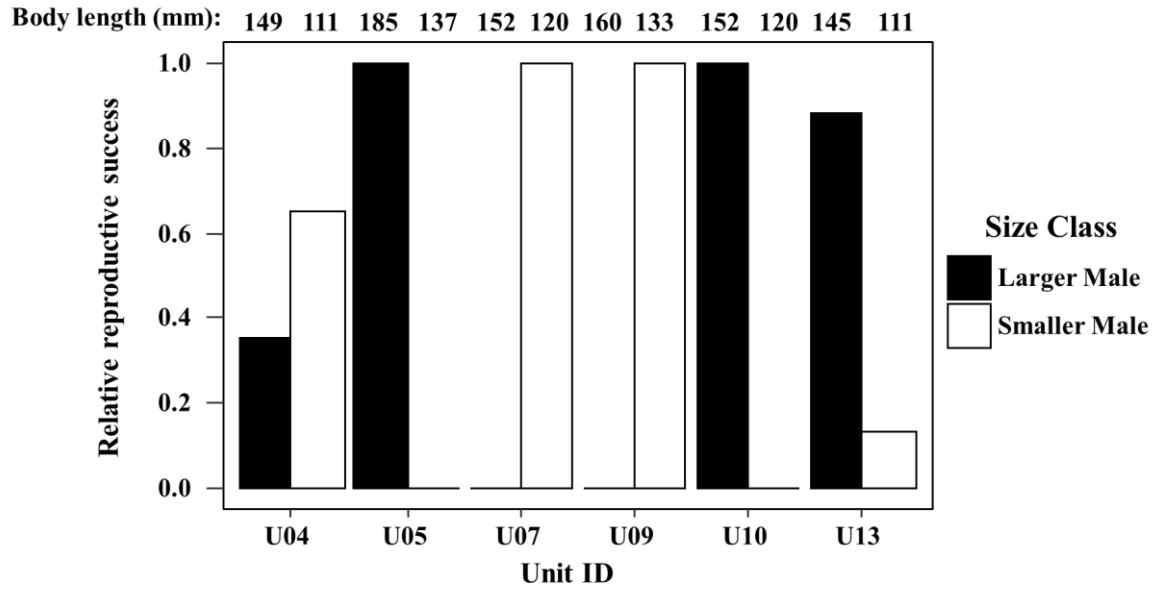
Fig. C1-2. Relative reproductive success of male bluehead chub in experiment units (EUs) conducted at Sixmile Creek. Size classes were shown in different color (black and white), and body size (mm) was shown on top of the figure.

5. References

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Appendix C2. Summary statistics and allelic diversity for eight microsatellite loci from the collected individuals in Shoal Creek (n= 667: 341 parents and 326 offspring). The number of alleles (k), observed (H_O) and expected (H_E) heterozygosity, mean polymorphic information content (PIC), significant departures from Hardy–Weinberg equilibrium (after sequential Bonferroni correction for multiple tests), and null allele frequency estimate (F(Null)).

Locus	k	H_O	H_E	PIC[1]	HW[2]	F(Null)
Ca5	31	0.882	0.909	0.902	NS	0.015
Nme25C8	7	0.507	0.504	0.469	NS	-0.003
Ca12	32	0.843	0.888	0.879	***	0.029
Ca11	24	0.904	0.893	0.883	NS	-0.006
Rhos5	26	0.886	0.898	0.889	NS	0.007
Rhos36	8	0.342	0.330	0.316	NS	-0.030
Ppro126	7	0.555	0.564	0.528	NS	0.011
Ca3	42	0.930	0.931	0.927	NS	0.000

[1]PIC: 0 – 0.29 (low); 0.3 – 0.59 (moderate); 0.6 ≤ (high).
[2]Significance with Bonferroni correction: ** < 0.01; *** < 0.001

Appendix C3. Pearson correlation tests based on the number of offspring assigned by each individual among different parameter setting of parentage analysis using COLONY2.

Sex		Model parameter*							
		M (0.7); F (0.6)	M (0.7); F (0.5)	M (0.7); F (0.4)	M (0.6); F (0.6)	M (0.6); F (0.5)	M (0.6); F (0.4)	M (0.5); F (0.6)	M (0.5); F (0.5)
Male	M(0.7); F (0.5)	0.77***							
	m (0.7); F (0.4)	0.97***	0.74***						
	m (0.6); F (0.6)	0.96***	0.83***	0.96***					
	M (0.6); F (0.5)	0.78***	0.99***	0.75***	0.85***				
	M (0.6); F (0.4)	0.74***	0.99***	0.73***	0.83***	0.99***			
	M (0.5); F (0.6)	0.95***	0.63***	0.97***	0.95***	0.66***	0.63***		
	M (0.5); F (0.5)	0.98***	0.73***	0.96***	0.94***	0.72***	0.70***	0.94***	
	M (0.5); F (0.4)	0.96***	0.70***	0.98***	0.92***	0.70***	0.68***	0.94***	0.97***
Female	M (0.7); F (0.5)	0.98***							
	M (0.7); F (0.4)	0.95***	0.92***						
	M (0.6); F (0.6)	0.97***	0.98***	0.92***					
	M (0.6); F (0.5)	0.98***	1.00***	0.93***	0.98***				
	M (0.6); F (0.4)	0.9***	0.9***	0.94***	0.88***	0.91***			
	M (0.5); F (0.6)	0.9***	0.9***	0.94***	0.89***	0.91***	1.00***		
	M (0.5); F (0.5)	0.97***	0.99***	0.92***	0.98***	0.99***	0.92***	0.93***	
	M (0.5); F (0.4)	0.65***	0.64***	0.79***	0.74***	0.65***	0.72***	0.72***	0.65***

*M: male; F: female. Probabilities of collected candidate fathers and mothers are shown in brackets.

***: *P*-value < 0.001

Appendix C4. The number of collected and recaptured individuals and recapture rate during the spawning season between May and June in 2017.

| Sexes	Sampling Date	Number of collected individuals	Number of recaptured individuals	Recapture rate
Male	MAY 3rd	28	16	0.57
	MAY 15th	18	13	0.72
	June 19th	8	6	0.75
	Mean*	-	-	0.68
Female	MAY 3rd	101	51	0.50
	MAY 15th	106	68	0.64
	June 19th	138	86	0.61
	Mean*	-	-	0.59

* Mean recapture rate was calculated based on recapture rates of three sampling occasions.

Appendix C5. A list of linear models to estimate body growth of bluehead chub. Models differed at the interval of capture-mark-recapture sampling occasion including January, April, May, and June.

Model	Parameter	Estimate	SE	t-value	P-value
January to April	Intercept	0.678	0.965	0.703	0.483
	Growth	1.051	0.012	91.271	< 0.000
April to May	Intercept	8.988	1.142	7.781	< 0.000
	Growth	0.963	0.013	74.023	< 0.000
May to June	Intercept	-1.660	1.118	-1.484	0.139
	Growth	1.072	0.012	88.898	< 0.000
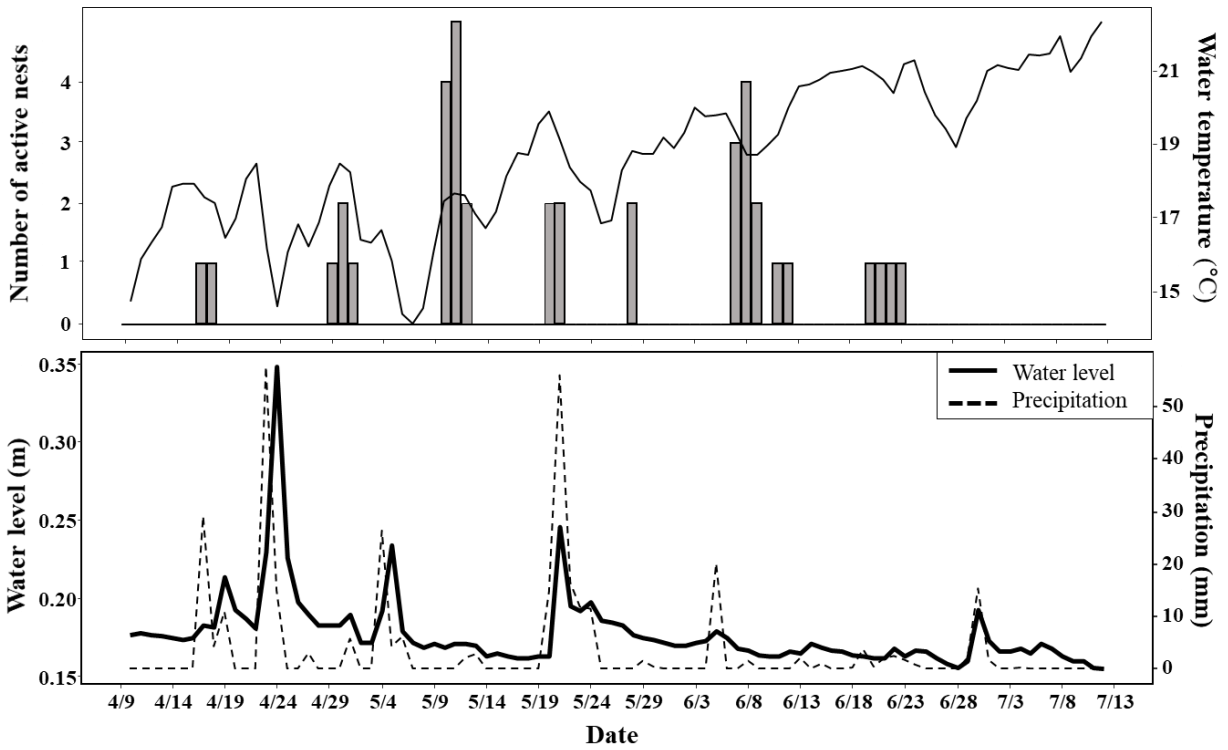
Appendix C6. Time series plots of the number of active nests and water temperature (top) and water level and precipitation (bottom) form April 9th to July 12th in 2017.
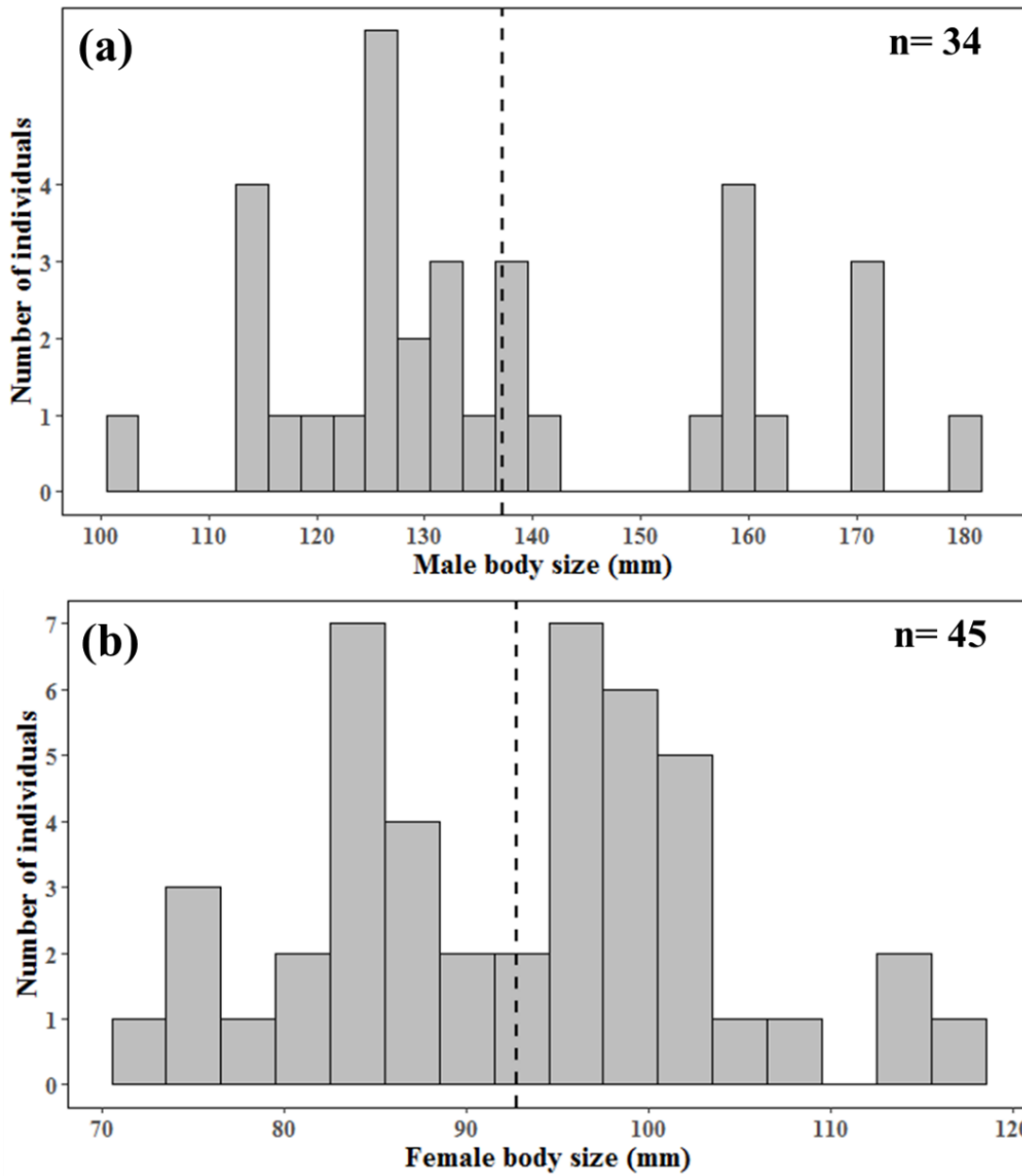
Appendix C7. Length frequency distribution of male (a) and female (b) bluehead chub. Black dashed lines indicate mean body size.

Appendix C8. List of candidate reproductive success models (competing models which Akaike weight ≥ 10% of the top-ranked model) by ascending values of Akaike's information criterion corrected for small sample size (AIC_c). Models differed in sex (male and female) and the combination of explanatory values. The global models are shown in bold.

Sex	Model	K	logLik	AIC_c	Δ AIC_c	Weight
	Largest nest size + Reproductive effort	4	-62.33	134.04	0.00	0.26
	Largest nest size + Reproductive effort + Movement	5	-61.77	135.68	1.63	0.12
	Body size + Largest nest size + Reproductive effort + Number of spawning	5	-62.07	136.29	2.25	0.09
	Largest nest size	3	-64.8	136.39	2.35	0.08
	Largest nest size + Reproductive effort + Number of spawning	5	-62.32	136.79	2.75	0.07
	Largest nest size + Movement	4	-63.72	136.82	2.78	0.07
Male	Largest nest size + Reproductive effort + Body size	6	-61.37	137.85	3.81	0.04
	Largest nest size + Number of spawning	4	-64.29	137.95	3.91	0.04
	Largest nest size + Reproductive effort + Number of spawning + Movement	6	-61.52	138.15	4.11	0.03
	Largest nest size + Reproductive effort + Movement + Timing	6	-61.69	138.48	4.44	0.03
	Largest nest size + Number of spawning + Movement	5	-63.25	138.65	4.60	0.03
	Body size + Largest nest size + Reproductive effort + Number of spawning + Movement + Timing	**9**	**-59.85**	**145.4**	**11.36**	**0.00**
	Body size	2	-65.51	135.31	0.00	0.22
	Body size + Largest nest size	3	-65.42	137.43	2.11	0.08
	Body size + Movement	3	-65.46	137.50	2.19	0.07
	Body size + Number of spawning	3	-65.49	137.57	2.25	0.07
Female	Body size + Timing	3	-65.5	137.58	2.26	0.07
	Body size + Effort	3	-65.51	137.61	2.3	0.07
	Body size + Effort + Largest nest size + Number of spawning + Movement + Timing	**7**	**-65.34**	**147.71**	**12.40**	**0.00**

Appendix C9. A list of the maximum nest size models (linear models) by ascending values of Akaike's information criterion corrected for small sample size (AIC_c). Models differed in the combination of explanatory variables.

Model	K	logLik	AIC_c	ΔAIC_c	Weight
Male number	3	-19.11	45.93	0.00	0.83
Male Size + Male number	4	-19.11	49.29	3.35	0.16
Null	2	-25.03	54.85	8.92	0.01
Male Size	3	-24.75	57.21	11.27	0.00

Appendix C10. List of candidate the female nest choice models by ascending values of Akaike's information criterion corrected for small sample size (AIC_c).

Model	K	logLik	AIC_c	ΔAIC_c	Weight
Male size + Male number	4	-56.52	124.11	0.00	0.69
Male size + Male number + Active nest size	5	-56.04	127.07	2.96	0.16
Male size + Active nest size	4	-58.19	127.45	3.34	0.13
Male size	3	-61.72	131.15	7.04	0.02