



Diverse reproductive patterns of Bluehead Chub (*Nocomis leptocephalus*) and their relationships with nest size and interactions with an associate, Yellowfin Shiner (*Notropis lutipinnis*)

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Abstract Nest construction by males is a common reproductive strategy in fishes, but little is known about intra-specific variation in nesting behavior and reproductive interactions with other species. In the southeastern USA, reproductive interactions between bluehead chub (*Nocomis leptocephalus*) and their nest associate, such as yellowfin shiner (*Notropis lutipinnis*), are mutualistic because the shiner requires chub nests to spawn but the chub also benefits from additional shiner eggs by reducing predation risk on their own eggs (i.e., dilution effect). As proxies for reproductive success of bluehead chub, we studied factors affecting their nest size variation and utilization by yellowfin shiner in three streams in the upper Piedmont region of South Carolina, USA. A total of 92 nests (71 spawned and 21 abandoned) were located, and we recorded spawning activity, nest size, number and size of male bluehead chub, number of yellowfin shiner, and environmental variables for 89 consecutive days in 2016. Path analysis showed that larger males spawned earlier in the season than smaller

males, and instances of pebble re-use for nest construction increased through the spawning season, which extended from April to June. Larger males more likely shared the same nest with each other than smaller males, and larger or more males constructed larger nests and attracted more yellowfin shiner. Nest size also increased when males re-used substrate materials from old nests, and nests built in this manner (36 nests) were equally prevalent as newly constructed nests (35 nests). This study suggests that complex mechanisms mediate host nest size and interactions with nest associates, and individual variation likely shapes dynamics of the mutualistic reproductive interaction.

Keywords Reproductive behavior · Mutualism · Leuciscidae · Individual variation · Fitness

Introduction

Fishes exhibit an array of parental care behavior for their offspring (Gross 2005). Nest construction, a form of parental care, is widely used among fishes (Barber 2013). Nest construction can serve two main functions for male fitness. First, males construct nests to protect eggs and larvae against predation and environmental conditions (e.g., high stream flows) (Suski and Ridgway 2007; Rushbrook et al. 2010). Thus, nest construction can maximize offspring survival by providing a shelter and stable environment (Chin-Baarstad et al. 2009). Second, nests provide information on male quality that influences mate choice by females (Blais

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et al. 2004; Wong et al. 2007). Accordingly, variation in nest size and design is a complex component of fish mating systems and consequently affects reproductive success (Wong et al. 2007; Barber 2013; Lehtonen et al. 2015).

Male body size is associated with the timing of spawning, social hierarchy, territoriality, nest quality, and hence reproductive success (Blais et al. 2004; Lindström 1992; Lehtonen et al. 2015). Larger males tend to spawn earlier and occupy better territories (Lindström 1992; Oliveira et al. 1999). They use aggressive behavior to secure nesting sites because dominance hierarchies are typically structured by body size (Lehtonen et al. 2015). In addition, larger males can construct higher quality (e.g., larger) nests because nest building is energetically costly by requiring males to collect building materials from surrounding areas (Lindström 1992). Thus, larger body size affords advantages for not only gaining access to more females (Blais et al. 2004), 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 (Sinervo and Zamudio 2001; Taborsky 1994). Additionally, 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 (Clutton-Brock 2002). 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 other 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 subsequently reproduction.

Factors affecting behavior and nest quality become further complicated when reproductive interactions occur with other species. Positive reproductive interactions (mutualism, commensalism, and facilitation) occur between many nest building species and ‘nest associates’, or 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 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). Previous studies have shown that associate species select nests guarded by larger males for spawning (Shao 1997). However, little is known about how a combination of factors, including nest quality (e.g., nest size) or nest sharing behavior (i.e., number of males on the same nest), operate to attract associates that determine reproductive success of nest building species.

Bluehead chub (*Nocomis leptoccephalus*) (hereafter, “chub”) is a suitable species to study intraspecific variation in nesting behavior and interspecific reproductive 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 are also common throughout the region (Wallin 1989, 1992; Marcy et al. 2005; Fig. 1). Chub prefer to spawn in particular mesohabitats (e.g. pool tails and slow runs; Peoples et al. 2014) and microhabitats (optimal depths and velocities; Bolton et al. 2015; Peoples et al. 2016). Single or multiple males construct a dome-shaped nest composed of pebble-sized substrates for spawning (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 utilize nesting resources (i.e., pebbles) from existing nests to build new ones later in the spawning season (Wallin 1989). Like some leuciscids, shiner are an obligate nest associate (Peoples and Frimpong 2016), and cannot spawn without a host species (Wallin 1992).

The goal of this study was to quantify factors affecting two key variables related to the reproductive success of chub: (1) nest size at the completion of reproductive activities (including parental care), and (2) nest utilization by shiner (shiner abundance on nests). We focused on these two response variables because (1) nest size is typically an indicator of reproductive success of males

in fishes (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 benefits egg survival of chub via the dilution effect (Johnston 1994b; Silknetter et al. 2019). Using a path analysis, we developed and tested a working conceptual hypothesis of causal relationships linking chub body size and spawning behavior (i.e., male number, nest material reuse) to nest size and shiner abundance.

Methods

To understand how male body size and nesting behaviors affect nest size and number of a mutualistic nest associate, we conducted daily observations of chub nests at three streams in northwestern South Carolina, USA. We developed a causal conceptual hypothesis depicting interrelationships between these independent variables and nest size/number of shiner (Fig. 2). We evaluated this hypothesis with the path analysis, a useful tool for quantifying complex relationships in which independent variables can also be dependent (endogenous), ultimately leading to prediction of terminal (exogenous) variables (i.e., nest size and number of shiner).

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. Streams were located within 4 km from each other in the same basin (Savannah River Basin), and 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 (mean wetted width = 2.8 m), and stream size increased in Todd Creek (34°45'15"N, 82°48'56"W; mean wetted width = 3.8 m) and in Sixmile Creek (34°45'36"N, 82°51'25"W; mean wetted width = 7.4 m). Lengths of the study stream were 1.6 km in Indian Creek, 0.8 km in Todd Creek, and 1.0 km in Simile Creek. Canopy density was highest at Indian Creek (mixed hardwoods) and was lowest at Todd Creek (shrubs). Pebble (16–64 mm) and gravel (2–16

mm), used by chub for nest construction, accounted for 47–49% of substrate across three streams (Table 1). 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 Creeks, but were much less abundant than yellowfin shiner.

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 with a unique number was placed on the nearest bank. 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-use of pebbles in nearby nests (Fig. 3). At each nest, water velocity ($\text{m}\cdot\text{s}^{-1}$) and depth (cm) were measured daily at upstream, downstream, right, and left locations 5 cm away from the edge of the nest.

Shiner spawn on chub nests, during which they display brilliant breeding colors (i.e., red body color with yellow fins) (Fig. 1), and both species spawn together (Wallin 1989, 1992). Thus, we considered a nest to be 'active' when more than one shiner with breeding colors were observed schooling or spawning on the nest. Once an active nest was located, observers stayed on the nearest bank for at least 15 min and used binoculars to record the spawning activity. Male chub have conspicuous external characteristics such as tubercles on their head and body colors (Jenkins and Burkhead 1994; Marcy et al. 2005), making them easy to be identified. We counted the number of male chub on each nest. The total length (TL) of shiner with breeding colors ranged 60–70 mm throughout the spawning season (*S. Kim*

Fig. 1 Photos of male bluehead chub (*Nocomis leptocephalus*) (top left) and yellowfin shiner (*Notropis lutipinnis*) (top right) with breeding colors, yellowfin shiner schooling on a chub nest (bottom left), and spawning of bluehead chub (bottom right). Photo credit: S. Kim



unpublished data). Using shiner as the size reference, we visually estimated TL of male chub and categorized 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. We 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 some 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 (cm) were recorded hourly by using loggers (Model U20L-004, HOBO Onset Computer Corp., Bourne, Massachusetts) at the downstream end of each study stream.

Variable definition

We defined two terminal variables in the path analysis; the maximum nest size and number of shiner using the nest (Table 2). First, nest size was calculated as the volume of an elliptical cone (m^3):

Fig. 2 A conceptual pathway diagram representing hypothesized relationships between abiotic and biotic factors (gray rectangles) and terminal variables (open rectangles) in the path analysis. Each arrow indicates hypothesized causality

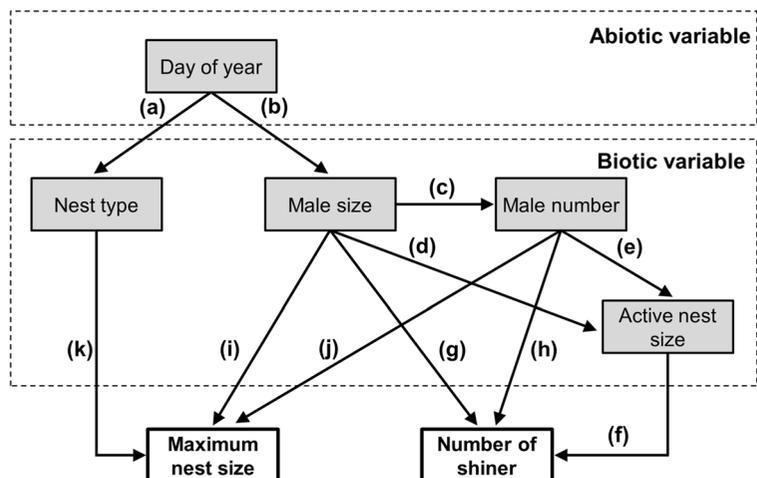


Table 1 Habitat characteristics of Indian Creek, Sixmile Creek and Todd Creek (South Carolina, USA) measured during the spawning season in 2016

Stream	Mean wetted width (m) ^a	Mean depth (cm) ^a	Substrate proportion ^b					Canopy cover
			SD	GV	PE	CO	BO	
Indian	2.8	16.6	0.31	0.21	0.28	0.15	0.05	High
Sixmile	7.4	32.1	0.40	0.25	0.22	0.08	0.05	Intermediate
Todd	3.8	24.7	0.06	0.14	0.33	0.22	0.25	Low

^a Mean wetted width and depth were based on measurements at transects, which were evenly distributed across study streams (Indian Creek: 48, Todd Creek: 32, Sixmile Creek: 20)

^b Substrate was visually estimated at transects. SD, sand (< 2 mm); GV, gravel (2–16 mm); PE, pebble (16–64 mm); CO, cobble (64–256 mm); BO, boulder (> 256 mm)

$$V(\text{volume, m}^3) = \pi ab \frac{h}{3} \tag{1}$$

where *a* and *b* were half of the length of major and minor axes, and *h* was the height of the nest. Male chub continue to move pebbles onto their nests for several days after spawning (Wallin 1989; Sabaj et al. 2000). Thus, we used the largest daily nest volume during the post-spawning period as the maximum nest size. Shiner abundance, an ordinal variable, was defined as the maximum number of individuals observed during a single daily observation. Shiner spawn on the same nest for more than one day (Kim and Kanno 2020), and different individuals could be present on different days. However, shiner individuals could not be uniquely identified and the maximum number of shiner individuals served as a measure of attractiveness and carrying capacity of a chub nest.

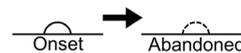
We defined four endogenous variables for male nesting behavior (Table 2). Male size was treated as an ordinal variable. Nesting locations or substrates are frequently re-used by male chub (Wallin 1989), and three nest types were identified during the field observations (Fig. 3). Nests that were constructed but abandoned

without signs of spawning were defined as ‘abandoned’; we observed nests constructed overnight and abandoned (evidenced by silt coverage and no activity) by the next morning. The ‘new’ nest type refers to instances when male chub constructed new nests at locations not previously used within the spawning season. In addition, male chub 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); in some cases, the nest expansion happened in a certain direction or only part of old nest, resulting in an elliptical nest shape. Typically, delineating the presently used part of an expanded nest was possible, and we measured the volume of this part. Nest type was included as a binary variable (new versus reused) (Table 2). The ‘abandoned’ nest type was excluded from the analysis because spawning was not observed. Active nest size was the average size of the nest across days when breeding color shiner were present. Active nest size differed from maximum nest size because the former was measured on days with spawning activities, but the latter was post-

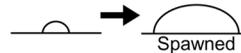
Fig. 3 Description of three nest types defined in this study. The re-used nest type consisted of two variants. Some expanded nests increased their size in a certain direction, resulting in an elliptical nest shape (i.e., expansion occurred only at a portion of the shaded space)

Nest type

1. Abandoned

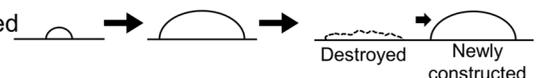


2. New



3. Reused

(1) Reconstructed



(2) Expanded

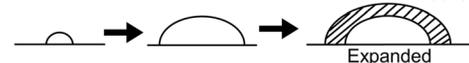


Table 2 List of variables considered in the path analysis to describe reproductive behavior of male bluehead chub and their interactions with yellowfin shiner at Indian Creek, Sixmile Creek and Todd Creek (South Carolina, USA) during the spawning season in 2016. Day of year was the only abiotic variable included

Category	Variable	Mean (SD) or Mode	Descriptions
Terminal	Maximum nest size ^a	0.026 (0.027)	Maximum nest size after spawning/construction (m ³)
	Number of shiner ^b	50–100	Maximum daily number of yellowfin shiner on active nest (ordinal variable)
Biotic	Male size ^b	15–18	Maximum male length (cm) on active nest (ordinal variable)
	Male number ^a	1.57 (0.91)	Number of male chub on the active nest
	Nest type	NA	New or re-used nest (binary variable)
	Active nest size ^a	0.015 (0.019)	Average nest size while active (m ³)
Abiotic	Day of year ^a	139.10 (17.88)	Sequential day number when nest was active
	Photoperiod ^a	833.17 (34.14)	Day length (minute)
	Precipitation ^a	6.01 (5.04)	Daily precipitation (mm)
	Water temperature ^a	18.67 (3.14)	Mean daily water temperature (°C)
	Water level ^a	0.25 (0.05)	Mean daily water level (cm)
	Depth ^a	28.71 (11.22)	Mean water depth at nest (cm)
	Velocity ^a	0.16 (0.05)	Mean water velocity at nest (m·s ⁻¹)

^aMean and standard deviation (SD)

^bMode

spawning when male chub continued to add pebbles to nests as a form of parental care.

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).

Hypothesis

We developed a conceptual path diagram to examine hypothesized direct and indirect relationships among variables, where each arrow indicates hypothesized causality (Fig. 2). The diagram represents tests for biotic variables (i.e., male body size and nesting behavior) playing pivotal roles in the potentially intricate network. We included only one abiotic variable (i.e., day of year) in the path diagram because it was significantly correlated with other abiotic variables including water temperature and level (Appendix S1A), which affect fine-scale spawning periodicity of chub spawning (Kim and Kanno 2020). In general, water temperature increased and water level decreased through time in the study period (Appendix S1). We hypothesized that day of year is important in the path analysis for two reasons. First, nest re-use would be more common later in the

in the conceptual diagram (Fig. 2), because its high correlation with other abiotic variables (photoperiod, precipitation, water temperature and water level) or lack of correlation with any biotic variables (depth and velocity) (Appendix S1)

spawning season (Fig. 2a), because males build only new nests and cannot “re-use” existing nests at the beginning of the spawning season. Second, larger males spawn earlier in many fishes, partly because smaller males may not yet be mature at the beginning of the season and require additional time for maturity (Lindström 1992; Oliveira et al. 1999; Fig. 2b).

Other causal relationships in the path analysis tested how biotic variables interacted with each other and linked them to two terminal variables of interest—maximum nest size and shiner abundance. We hypothesized that larger males would be more aggressive and occupy nests by themselves (Lehtonen et al. 2015), but smaller males would be more likely to share nests because their size disadvantage motivates them to cooperate (Taborsky 1994; Díaz-Muñoz et al. 2014; Fig. 2c). Larger males (Fig. 2d) or more males (Fig. 2e) would build larger active nests, which would in turn attract more shiner (Fig. 2f) if active nest size is indeed an important criterion for shiner in selecting among nests. Alternatively, active nest size may not be important for shiner, but instead the number of shiner may be directly linked to size (Fig. 2g) or number (Fig. 2h) of male chub. Finally, we hypothesized that the maximum nest size would similarly be affected by male size (Fig. 2i) or number (Fig. 2j) because nest size increases when larger

males use more or coarser substrates, or when more males provide cooperative parental care. Re-using nesting resources would also increase nest size if nest building with pebbles from elsewhere is energetically demanding (Fig. 2k). A pathway from the nest type to the number of shiner was not established because shiner were not likely to distinguish how nests were built (i.e., new or reconstructed).

Statistical analysis

We used the path analysis to quantify factors affecting variation in the maximum nest size and number of shiner on each nest (Fig. 2). The path analysis is a specific case of structural equation modeling with no latent variable (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 models (i.e. sub-models or paths), the path analysis is unique in providing inferences on specific paths (regression coefficients, b) as well as the plausibility of the global model. The path analysis also allows for the estimation of total effects as the product of all paths leading to a given response.

Prior to analysis, numeric 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. We analyzed data from three study streams simultaneously in the path analysis by including stream identities as dummy variables (a combination of ones and zeros to model a categorical variable). Two binary dummy variables (i.e., D1 and D2) were included in pathways for both terminal variables to model the effects of Sixmile Creek (D1 = 1, D2 = 0) and Todd Creek (D1 = 0, D2 = 1), relative to Indian Creek (reference stream: D1 = 0, D2 = 0). Dummy variables were only included for terminal variables because the maximum nest size differed among streams (Results). Because Pearson correlation tests of variables in the path analysis were similar whether streams were analyzed individually or together (Appendix S1), we posited that the use of dummy variables will provide a superior approach by synthesizing data across streams while accounting for variation among streams.

Each hypothesized pathway was tested based on statistical significance set at $\alpha = 0.05$, and non-significant paths were removed sequentially, beginning the one with the lowest effect size, to develop a

parsimonious model that passed model fit diagnostics. The path analysis was fit using a 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 (RMSEA, 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 structures 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).

Results

Environmental conditions

Water temperature increased gradually during the study period (April 4th – July 1st, 2016). Mean water temperature in Indian Creek (mean = 17.7 °C, SD = 2.7) was colder than Sixmile Creek (mean = 19.1 °C, SD = 3.3) and Todd Creek (mean = 19.2 °C, SD = 3.2). Mean photoperiod was 833 min (SD = 34) and increased gradually until early June and then remained stable until July 1st. Precipitation occurred on 18 days, and the mean value across these days was 6.01 mm. Water level declined generally during the study period but was influenced by precipitation. Flow condition in Indian Creek (mean = 18.3 cm, CV = 5.4) was more stable over time than other streams (Sixmile Creek, mean = 29.5 cm, CV = 9.3; Todd Creek, mean = 28.2 cm, CV = 7.6). Mean water depth and velocity at nests were 28.7 cm (SD = 11.2) and 0.16 m·s⁻¹ (SD = 0.05), respectively (Table 2).

Spawning activities

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 3). Of possible nest types, 35 nests were recorded as ‘new’, 36 were ‘reused’, and 21 were ‘abandoned’ (Table 3, Fig. 3). Nests were attended by a single or multiple males (up to four males, Fig. 4). The number of males on nests was not significantly different among streams

(Kruskal-Wallis Rank Sum test: $\chi^2_2 = 5.67$, $p = 0.06$). Mode of male size across study streams was 15–18 cm (Table 2), and male body size did not differ by stream (χ^2_4 : $p = 0.09$ – 0.29 ; Fig. 4). The mode of daily number of shiner on active nests across study streams was 50–100 individuals (Fig. 4), and it was not significantly different among streams (χ^2_6 : $p = 0.21$ – 0.24). The mean size of active nests was 0.015 m^3 , and the mean maximum size of the nest was 0.026 m^3 (Table 2). Maximum nest size differed significantly among streams (one-way ANOVA: $F_{2, 68} = 37$, $P < 0.001$; Fig. 4). Specifically, nests in Sixmile Creek (mean = 0.061, SD = 0.035) were larger than those in Indian (mean = 0.015, SD = 0.008) or Todd Creek (mean = 0.016, SD = 0.011; Tukey's honestly significant difference post hoc test: $p < 0.001$), but there was no significant difference between Indian and Todd Creek (Tukey's honestly significant difference post hoc test: $p = 0.98$).

Path analysis

The global path model fit the data poorly (χ^2_{33} $p < 0.001$, CFI = 0.57, RMSEA = 0.27) and contained uninformative paths (Appendix S2). After removing three uninformative paths, beginning the one with the weakest effect size, we identified a stable final model with adequate global fit and all paths were significant. The final path model provided a good fit to the data (χ^2_{25} $p = 0.75$; CFI > 0.99; RMSEA < 0.001), and retained 8 paths (Fig. 5). Additionally, stream identities (dummy variables: D1 = Sixmile Creek, D2 = Todd Creek) on each terminal variable were not significant (maximum nest size: D1, $b = 0.06$, SE = 0.19, $p = 0.78$; D2, $b = 0.02$, SE = 0.21, $p = 0.94$; number of shiner: D1, $b = 0.04$, SE = 0.26, $p = 0.89$; D2, $b = 0.03$, SE = 0.24, $p = 0.90$; Appendix S2).

As hypothesized, larger males spawned earlier than smaller males ($b = -0.29$, SE = 0.12, $p = 0.01$; Fig. 5), and nests were frequently re-constructed later in the spawning season ($b = 0.18$, SE = 0.06, $p = 0.001$). Contrary to our hypothesis, larger males were more likely to spawn together than smaller males ($b = 0.41$, SE = 0.11, $p < 0.001$). Both terminal variables were influenced by complex pathways. The number of shiner increased when larger males ($b = 0.29$, SE = 0.11, $p = 0.007$) and more males ($b = 0.51$, SE = 0.14, $p < 0.001$) occupied nests. Active nest size in the conceptual diagram (Fig. 2) was not retained as a driver of shiner abundance in the global model (Fig. 5), meaning that size and number of

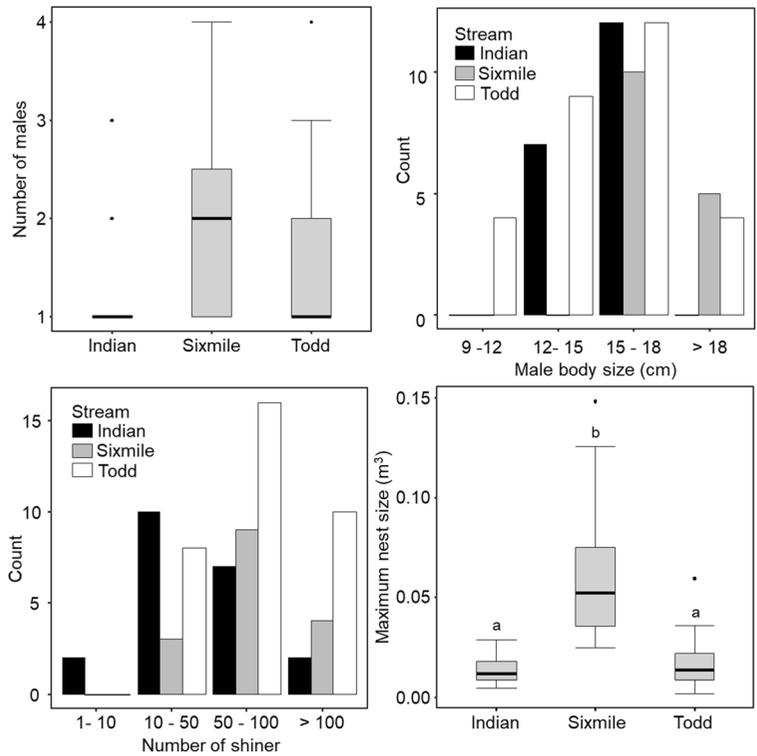
bluehead chub affected number of shiner irrespective of active nest size. Maximum nest size increased when larger males attended nests ($b = 0.30$, SE = 0.09, $p = 0.001$), more males attended nests ($b = 0.51$, SE = 0.14, $p < 0.001$), and nests were reconstructed using pebbles from existing nests ($b = 0.40$, SE = 0.16, $p = 0.01$). In addition, male size indirectly influenced both terminal variables through its effects on male number (Fig. 5). As a result, the total effect of male size was 0.51 (direct = 0.30, indirect = 0.21) on the maximum nest size and 0.46 (direct = 0.29, indirect = 0.17) on the number of shiner, equivalent to direct effects of the number of males on both terminal variables (maximum nest size, $b = 0.51$; number of shiner, $b = 0.42$).

Discussion

Our findings suggested that the maximum nest size and shiner number (i.e., proxies of chub reproductive success) were influenced by a multitude of factors. Similar to previous studies, male body size was an important factor to explain variation in nest size (Lindström 1992; Oliveira et al. 1999; Lehtonen et al. 2007, 2015), and nest sharing behavior (cooperative parental care) and nest building mechanisms (new or reconstructed) affected nest size (Taborsky 1994; Díaz-Muñoz et al. 2014). Our study also provided novel insights by demonstrating that the male size and number of a host species (chub) influenced interactions with a nest associate. Diverse reproductive patterns of chub and their consequences on nest size and inter-specific interactions suggest that individual variation likely plays a key role in the evolution of reproductive mutualism in this system (Johnston 1994b; Peoples and Frimpong 2016; Silknetter et al. 2019).

The number of males sharing a nest and nest re-use had the strongest direct effects on the maximum nest size. Male chub invest a large amount of energy to care for their eggs by moving thousands of pebbles after spawning (Wallin 1989, 1992). Thus, building nests together and sharing nests for spawning may alleviate their individual effort. As hypothesized, 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 (Lindström 1992; Gross 2005). This behavior may be particularly important for smaller males that more likely spawned later in the season when

Fig. 4 Variation in the number of males on the nest (top left), male body size (top right), number of shiner on the nest (bottom left), and maximum nest size (bottom right) among three study streams. Boxplots show median, 25th and 75th percentiles in boxes, minimum and maximum values with whiskers. Alphabetic letters on the maximum nest size shows statistical differences based on the Tukey’s honestly significant difference *post-hoc* test following a one-way analysis of variance ($P < 0.05$)



they could take advantage of existing nests. The reconstructed nest type was similarly observed by Wallin (1989). In our study, approximately half of nests were reused, indicating that this behavior is more common than previously thought. We further expanded knowledge on fine but discernable differences in reused nest types by documenting that males not only reconstructed nests anew but also added pebbles on top of existing nests (i.e., expanded type). We cannot exclude the possibility that pebbles and gravels were limited resources (McManamay et al. 2010; Peoples et al. 2014; Bolton et al. 2015), and male chub spawned

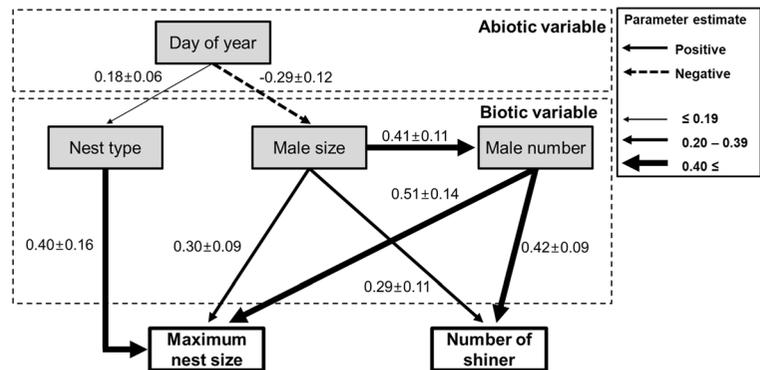
together and re-used nests by necessity, not but by choice. However, 47–49% of substrates were available as pebbles and gravels (Table 1) and suitably-sized substrates did not appear particularly limited across study streams.

The role of nest construction has been mainly described as a means to attract mates and care for young (Gross 2005; Sinervo and Zamudio 2001), but many studies have also shown nest association can benefit nest building species (Johnston 1994b; Peoples and Frimpong 2016). Based on the total (direct and indirect) effect sizes, male chub size and number were key factors

Table 3 Summary of nest types at Indian Creek, Sixmile Creek and Todd Creek (South Carolina, USA) during the spawning season in 2016

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

Fig. 5 The final path model. All arrows are significant ($P < 0.05$). Each arrow is shown with mean coefficient \pm standard error and the thickness of arrows indicates the strength of the coefficient. Solid and dashed arrows indicate positive and negative relationships, respectively



for attracting shiner to nests. Interestingly, the size of the active nest did not significantly affect the number of shiner. This indicated that the large size of the active nest alone was not sufficient to attract more shiner, but the number and quality (i.e., body size) of male chub were more important determinants for 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 had stopped attending or artificially constructed nest-like mounds. Just like shiner eggs afford a dilution effect to chub, reducing the likelihood of predation on chub eggs, shiner similarly may benefit by laying their eggs where more chub eggs are present, which would favor a reproductive system where both species spawn together.

We could not separate the influences of male size and number on the two terminal variables because they were highly correlated with each other (Fig. 5). Contrary to our hypothesis, larger male chub were more likely to spawn together, which was surprising given large males were aggressive with each other in previous studies (Maurakis et al. 1991). Building and using a nest together appeared to be a form of male cooperation (Díaz-Muñoz et al. 2014), but social hierarchies were also present on the nest. Specifically, the largest male usually dug a spawning pit at the upstream area of a mound (presumably the most ideal location), and other males made their own pits elsewhere, as observed by Maurakis et al. 1991; Sabaj et al. 2000. These observations suggest that the costs and benefits of cooperation differ at the whole-nest and within-nest scales. Several males may engage in congregating and displaying behaviors, available resources, and quality nests to attract more females to their nest (Clutton-Brock 2002; Díaz-

Muñoz et al. 2014). Such benefits of nest sharing may exceed the cost of aggressive interactions among males through more parental effort (i.e., maximum nest size) and dilution effect (i.e., number of shiner). Because the total effect of male size on the two terminal variables was similar to the direct effect of male size, our data do not provide a definitive conclusion on the relative importance of male size and number. An experimental approach would be helpful for revealing these effects.

This study was based on detailed observations of chub nests and spawning activities during the entire spawning season, but it had some limitations. First, we 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), but we were not confident about enumerating the number of females on a nest without monitoring the nest continuously over the entire period when the nest was active. Second, we used the maximum nest size and number of shiner as surrogates of male reproductive success, but we did not directly measure reproductive success (e.g., number of offspring that hatched successfully). Lastly, our study streams had only one dominant nest associate (i.e., yellowfin shiner), but multiple nest associates use bluehead chub nests elsewhere (Johnston and Page 1992; Johnston 1994a; Peoples et al. 2011). Inter-specific reproductive interactions are likely even more complex in those streams. Further research is warranted to link reproductive behavior to fitness of both sexes of chub, which will require unique identification of individuals to enable more direct measures of reproductive success for both chub and their nest associates.

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