

Linking male reproductive success to effort within and among nests in a co-breeding stream fish

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Abstract

Nest construction is an energetically costly behavior displayed by males in many taxa. In some species, males construct nests and co-breed with other males and they may construct multiple nests in a breeding season. However, little is understood about how allocation of effort within and among nests affects male reproductive success. We characterized reproductive effort of male bluehead chub (*Nocomis leptoccephalus*), a nest-building stream fish, in an entire breeding season in a small stream in South Carolina, USA. By using automated monitoring methods on 18 nests, we recorded time spent by 34 males during the spawning season in 2017. We then linked effort within and among nests to reproductive success, measured by the number of offspring assigned genetically to each male. A Bayesian hierarchical analysis showed that larger males spent more time constructing and maintaining a given nest, and consequently were more reproductively successful than smaller males on the same nest. Combined with aggressive behavior displayed by larger males toward smaller males, this finding suggested that reproductive effort, including agonistic interactions within nests was a determinant of reproductive success. In addition, larger nests constructed by more males led to higher male reproductive success, suggesting the importance of cooperative behaviors. Number of nests that males constructed, a measure of effort across nests, was not a predictor of reproductive success, further supporting that reproductive success varied among nests due to nest size. Our study showed that male reproductive success was determined by both aggressive and cooperative behaviors in a co-breeding species.

KEYWORDS

alternative tactics, competition, cooperation, fitness, *Nocomis*

1 | INTRODUCTION

Reproductive success (e.g., number of offspring) shapes individual life history and population genetics and dynamics (Hedrick, 2005; Merilä & Sheldon, 2000). Male reproductive success often depends on reproductive effort because males compete with each other for limited resources such as mates and breeding territories (Andersson,

1994; Emlen & Oring, 1977; Reynolds, 1996). However, males cannot indefinitely expend reproductive effort. Thus, allocation of current and future efforts, such as investment in current reproduction as opposed to survival and growth, determines lifetime reproductive success (Dubuc et al., 2014; Galimberti et al., 2007; Tarwater & Arcese, 2017). The effect of male efforts on reproductive success has been well documented in many animal taxa, whereas little is understood

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about how males allocate reproductive effort for successful reproduction within a breeding season (Andersson, 1994; Dubuc et al., 2014; Metz et al., 2007; Reynolds, 1996).

Males exhibit diverse reproductive tactics, and males of some species co-breed by sharing resources and territories (Díaz-Muñoz et al., 2014; Taborsky, 1994, 2009). Social hierarchy is not readily evident in co-breeding males (Balshine et al., 2001; Díaz-Muñoz et al., 2014; Stiver et al., 2013). Thus, the relationship between reproductive success and behavior is elusive compared with cooperative males who form a social structure or partition their roles (Hellmann et al., 2020; Koenig & Walters, 2015; Olson & Blumstein, 2010; Taborsky, 2001). Such co-breeding behavior is observed in some nest-building species, in which males construct nests to attract females and provide parental care of the young in aquatic and terrestrial animals (Barber, 2013; Gross, 2005; Soler et al., 1998). As nest construction is energetically costly, males typically own their nests and spend much effort on nest construction and defense (Bose et al., 2018; Lehtonen et al., 2007; Metz et al., 2007; Soler et al., 1998). But in some species, males co-breed with other males by building and sharing nests (Díaz-Muñoz et al., 2014; Gross, 1996; Taborsky, 2009). This behavior is adaptive because males in a certain group may have a better chance to increase reproductive success (Balshine et al., 2001; Riehl, 2012; Sherley, 1990; Stiver et al., 2013; Theuerkauf et al., 2009). In addition, males of some species build multiple nests sequentially in a reproductive season (Friedl & Klump, 2000; Sumasgutner et al., 2016). Co-breeding males displaying this behavior must allocate their reproductive effort within and among nests. However, the consequences of co-breeding behaviors on male reproductive success are little known because characterizing reproductive effort and behavior of multiple males across nests is challenging in the wild.

Several behavioral mechanisms are potentially available for co-breeding males to increase reproductive success over a single breeding season. First, males may invest more effort in constructing and maintaining a nest than other co-breeding males (Evans, 1997; Sherley, 1990). Intensive efforts on a given nest may lead to successful reproduction in some cases (Evans, 1997; Theuerkauf et al., 2009) but not in others (Sherley, 1990; Szentirmai et al., 2005). Second, co-breeding males may increase reproductive success by building nests of certain characteristics. For instance, males with larger nests result in higher reproductive success in birds and fishes (Barber, 2013; Bose et al., 2018; Lehtonen et al., 2007; Soler et al., 1998). Finally, males may build multiple nests across a breeding season to increase reproductive success (Friedl & Klump, 2000; Sumasgutner et al., 2016). This is a bet-hedging strategy when some nests fail to produce the young (Gross, 1996; Koenig & Walters, 2015), and co-breeding may facilitate such a strategy as individual males need not expend as much effort as constructing nests alone. Males may use single or multiple behaviors to allocate reproductive efforts within and among nests, but we know little about the relative importance of these behavioral mechanisms as determinants of reproductive success in co-breeding males.

Bluehead chub (*Nocomis leptoccephalus*) provide a unique opportunity to study whether allocation of nesting efforts increases reproductive success within a breeding season. Bluehead chub are freshwater

fish native to streams in the Atlantic slope of the southeastern USA (Lachner, 1952; Marcy et al., 2005). Males of bluehead chub construct dome-shaped nests by moving thousands of pebbles individually by mouth to keep nests free of silt as a form of parental care (Maurakis et al., 1992; Wallin, 1989, 1992). Multiple males share and spawn on the same nest as co-breeders, although some males construct nests alone (Kim, Peoples, et al., 2020; Sabaj et al., 2000; Wallin, 1989). When males co-breed on the same nest, each male occupies a different nest location and excavates a small spawning pit where mating occurs (Sabaj et al., 2000; Wallin, 1989). Males invest intensive efforts to guard their spawning pits until they finish spawning; thus, sneaking behavior, such as fertilizing eggs when guarding males are absent, is not observed (Kim, Pregler, et al., 2020). Larger males are likely to occupy better positions for spawning, but they still allow smaller males to spawn on their nests by displaying mutual tolerance (Kim, Peoples, et al., 2020; Wallin, 1989). Body size and mating with more partners lead to higher reproductive success in female bluehead chub (Kim, Pregler, et al., 2020). Bluehead chub spawn between spring and early summer, while spawning occurs periodically at intervals up to one week and multiple nests are observed on the same days across local streams (Kim & Kanno, 2020; Wallin, 1989). Males typically spend up to 4 days on nests for construction and spawning (Kim, Peoples, et al., 2020; Wallin, 1989). In addition, nests constructed by bluehead chub are used simultaneously by other species (i.e., nest associates), which do not contribute to nest building. Such inter-specific relationship is mutualistic because bluehead chub provide nests to nest associates and benefit from the presence of eggs from other fishes to reduce mortality of their own eggs by predators (Cashner & Bart, 2010; Johnston, 1994). These unique reproductive behaviors of bluehead chub can serve as an excellent model system to examine how allocation efforts within and among nests within a single spawning season mediate reproductive success of co-breeding males.

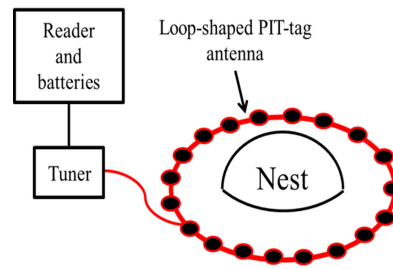
In this study, we address this knowledge gap by characterizing co-breeding behaviors of uniquely marked male bluehead chub in an entire breeding season and their reproductive success. We investigated whether male reproductive success could be explained by (1) relative effort on nest construction and parental care among co-breeding males at each nest, measured by time spent by each co-breeding male at the nest, (2) nest size, an index of effort to move and arrange pebbles, and (3) number of nests males constructed in a breeding season. Behavior of co-breeding males was quantified using an automated tracking system deployed at nests, and reproductive success of males was inferred by the number of offspring assigned genetically to them through parentage.

2 | MATERIALS AND METHODS

2.1 | Hypotheses

We test three plausible behavioral mechanisms to link male reproductive success to effort within and among nests. First, we hypothesize that co-breeding males do not spend an equal amount of reproductive

FIGURE 1 Design of the loop-shaped passive integrated transponder (PIT) antenna to monitor male bluehead chub on nests (left). Photo showing two male bluehead chub (yellow arrows) and yellowfin shiner (red individuals) spawning on the nest (right)



effort on a given nest, and investing more effort leads to successful reproduction. Larger males typically dominate smaller males on the same nest in nest-building fishes (Hellmann et al., 2020; Taborsky, 1994). Thus, we expect that reproductive effort and hence success increases with male body size. Our second hypothesis is that males reproducing on larger nests are more successful than those on smaller nests. Nest size increases with the size and number of males constructing the nest, and larger nests are visited by more bluehead chub females and nest associates (Kim, Peoples, et al., 2020; Kim, Pregler, et al., 2020). Males would benefit from increased encounter rates with females, and the presence of more eggs of nest associates to dilute predation impacts on their own eggs (Kim, Peoples, et al., 2020; Silknetter et al., 2019). Third, we hypothesize that constructing more nests increases male reproductive success. Spawning of bluehead chub occurs at intervals of several days in a breeding season (Kim & Kanno, 2020), allowing males to construct nests several times in a season. Males stop nest construction and maintenance when abiotic conditions become unfavorable, and nests may be damaged or destroyed by high stream flows due to precipitation (Kim & Kanno, 2020). This stochastic factor in male reproductive success would favor investment of reproductive effort across nests at different times in a breeding season.

2.2 | Study area

This study was conducted in Shoal Creek (34°48'12" N, 82°47'02" W) located in the Savannah River Basin of South Carolina, USA (Appendix S1). We chose an 880-m-long study area (mean wetted width = 3.1 m), where bluehead chub were abundant. Their abundance decreased farther upstream due to small stream size and mixed grazing and residential land use, and farther downstream due to lentic conditions created by beaver (*Castor canadensis*) dams. Thus, we assumed that fish would stay within the study area over a spawning season due to their limited movement. Two nest associates were present in Shoal Creek: yellowfin shiner (*Notropis lutipinnis*) and rosyface chub (*Hybopsis rubrifrons*).

2.3 | Field sampling

Field sampling protocols are detailed in Kim, Pregler, et al. (2020). Briefly, we marked bluehead chub males with passive integrated

transponder (PIT) tags and deployed PIT antennas around nests to record the amount of time each male spent on each nest throughout a breeding season. Later, we collected young-of-the-year (YOY) fish to assign parentage to males using microsatellite markers.

Adult bluehead chub were collected with backpack electrofishing twice before the breeding season (January and April), and three times during the breeding season between May and June 2017. Fish were measured for total length (mm), and genetic samples were taken non-lethally by clipping anal fins. Sex was noted based on secondary characteristics such as tubercles on their head and body colors (Marcy et al., 2005). Individuals ≥ 60 mm in length were marked by inserting 12-mm half duplex (HDX) PIT tags (Oregon RFID) into the abdominal cavity. We marked 64 males, and a total of 622 females and immature individuals for which sex could not be determined visually.

To monitor nest use by males throughout a breeding season, we visited the study stream daily for 103 consecutive days from 1 April to 12 July 2017. Mean daily water temperature ranged 12.8–22.3°C (mean = 18.3) during this period, and water level was affected by precipitation (Appendix S2). Nests were readily identifiable because male bluehead chub construct conspicuous pebble mounds at shallower runs and yellowfin shiner spawn in large numbers and display brilliant breeding colors (Figure 1). Once a nest was located, a loop-shaped PIT antenna made of copper wire (2.59 mm in diameter) was deployed around the nest. Key reproductive behaviors, such as encounters with females and agonistic interactions among males, occur primarily when bluehead chub spawn on nests, although males often maintain nests after spawning (Maurakis et al., 1992; Sabaj et al., 2000; Wallin, 1989). Thus, we recorded male identity and duration that each male spent on each nest until spawning ceased to measure reproductive effort. We monitored up to 6 nests simultaneously using 3 multi-antenna HDX readers (Oregon RFID). A total of 21 nests were located and we monitored 18 nests between 29 April and 23 June, whereas antennas could not be deployed on 3 nests due to technical issues. During the study period, the antennas detected 49 out of the 64 marked males and 277 out of 622 females. We used these data to quantify the relative reproductive effort of each male per nest, defined as the number of antenna detections for a male divided by the total number of detections of all males that spawned on the nest. The proportion of antenna detections on the nest by each male was correlated positively with the hourly-scale proportion of time spent (Pearson's correlation test: $r = .78$, $p < .001$). Therefore,

we used the proportion of antenna detections as an index of the relative duration of time spent. Fifteen of the 49 males detected by antennas visited nests only briefly and their reproductive success could not be genetically confirmed (Kim, Pregler, et al., 2020). We retained the remaining non-transient 34 males who were detected by antennas. All nests were measured daily for their size (length, width and height), and we estimated nest size based on the largest daily measurement using the volume of elliptical cone (mean = 0.047 m³, $N = 18$, range = 0.015–0.116).

To infer male reproductive success, YOY were collected with backpack electrofishing between 25 August and 10 September 2017. Fin clips were taken for genetic analysis. We sampled YOY in the 880-m study area and an additional 100-m section immediately downstream to collect individuals that might have drifted downstream. However, we did not collect a single YOY individual in the downstream section. A total of 326 YOY were collected ranging in total length between 16–49 mm (mean = 31 mm).

2.4 | Genetic parentage assignment

A set of 8 polymorphic microsatellite loci was used for the genetic parentage assignment (Cushman et al., 2020). We genotyped all 49 males and 277 females and immature individuals detected on nests by antennas and 326 YOY for parentage analysis. Females were included to increase accuracy in parentage assignment in program COLONY2 version 2.0.6.5 (Jones & Wang, 2010). Paternity was assigned when the assignment probability exceeded 95%. Analytical details can be found in Kim, Pregler, et al. (2020).

2.5 | Statistical analysis

Three sets of regression analyses were conducted to address our study objectives by (1) linking reproductive effort (i.e., proportion of detections) with male body size, (2) comparing effects of size and number of males on nest size, and (3) evaluating how reproductive effort within and among nests affected male reproductive success. Predictors were centered and standardized (mean = 0 and SD = 1), and all analyses were conducted in R version 4.0.1 (R Development Core Team, 2020).

We tested whether larger males spent more reproductive effort than smaller males on a given nest, using a linear mixed-effects model to account for variation among nests. The response was the proportion of antenna detections for each individual at each nest, and the predictor was male body size. For the random effect, we included a random intercept and slope to account for variation in male body size across nests. Additionally, nest-specific intercepts and slopes were characterized by conditional normal distributions to calculate overall mean and variation. The model was fit using the package *lme4* (Bates et al., 2015), and statistical significance of the overall mean effect of body size on reproductive effort was examined at $\alpha = .05$ using the package *lmerTest* (Kuznetsova et al., 2017).

We examined the effects of body size and number of males on nest size using a linear regression model. The response was nest size, and the predictors were the mean size of males and number of males detected on each nest. Male body size is an important factor for constructing larger nests in nest-building fishes (Bose et al., 2018; Kim, Peoples, et al., 2020; Lehtonen et al., 2007). Thus, we included male body size to assess the relative importance of size and number of males in nest size. Statistical significance was identified based on $\alpha = .05$.

We investigated how reproductive effort within and among nests affected male reproductive success using a hierarchical regression model. This analysis tested whether variation in reproductive success can be explained by male body size, and size and number of nests that males constructed. Reproductive success, or number of offspring, of male i ($i = 1, \dots, 34$) was modeled as $y_i \sim \text{Poisson}(\lambda_i)$, where the heterogeneity in intensity was characterized by:

$$\log(\lambda_i) \sim \text{Normal}\left(x_i \beta + \sum_{j=1}^{18} w_{ij} \alpha_j, \sigma^2\right). \quad (1)$$

In Equation (1), body size of male i was denoted by x_i with its fixed effect β , α_j referred to random effects of nest j ($j = 1, \dots, 18$) on which male i spawned, and the conditional variance of intensity, $\log(\lambda_i)$, was denoted by σ^2 . Because random effects of nests were accounted for, we tested whether larger males were more reproductively successful than smaller males on a given nest via the fixed effect β . Furthermore, size of nest j was included to explain nest-level variation in male reproductive success as:

$$\alpha_j \sim \text{Normal}\left(\gamma_0 + z_j \gamma_1, \sigma_\alpha^2\right) \quad (2)$$

where γ_0 was the intercept and γ_1 was the fixed effect of nest size z_j . The w_{ij} in Equation (1) expressed the membership of individual i on j^{th} nest, weighted by reproductive effort. Therefore, $w_{ij} = 0$ for a nest j on which individual i did not spawn, otherwise $0 < w_{ij} \leq 1$. This approach explicitly considered nest-level variation in male reproductive success due potentially to nest size, while assuming that reproductive success among males on a given nest is proportional to effort expended by each male on that nest. Thus, the hierarchical model tested whether reproductive success was affected by the size and number of nests males constructed and within-nest reproductive effort. The sum of $w_{ij} \alpha_j$ across J nests in Equation (1) indicated the accumulation of nest random effects weighted by reproductive effort for each male i . We evaluated whether the cumulative nest random effects increased due to the number of nests that each male visited or cumulative sum of nest size he visited, using post hoc linear regression.

The hierarchical model was fit to our data in R using a MCMC algorithm with Gibbs updates for all parameters except for λ_i , which had full-conditional distributions that were not conjugate (Hooten & Hefley, 2019). We used random walk Metropolis-Hastings updates for λ_i , a standard practice when fitting Bayesian generalized linear models (Metropolis et al., 1953). Relatively flat priors (diffuse priors) were used for all parameters. We ran the MCMC algorithm for

100,000 iterations and omitted the first 10,000 as burn-in. Effects of male body size (β) and nest size (γ) on reproductive success were judged statistically significant if 95% marginal posterior credible intervals (CI) did not overlap zero.

3 | RESULTS

We monitored 18 nests between 1 April and 12 July 2017, and a total of 49 males were detected by PIT antennas. Based on the frequency of detections by PIT antennas, 34 of 49 males were deemed non-transient males who constructed and spawned on nests in the study area.

Reproductive success was highly variable among males. Of the 34 non-transient males detected on the 18 nests, 15 males were genetically assigned to 169 YOY out of 326 genotyped (52%). The number of YOY assigned (i.e., reproductive success) ranged 1–33 among males (mean = 10). The 15 males with reproductive success ranged from 115 to 172 mm (mean = 141), and their body size did not differ significantly from the other 19 males without confirmed reproductive success (mean = 135 mm; range = 103–180) (t -test: $t = -1.04$, $df = 31$, $p = .31$). In general, larger males were detected at nests earlier in the spawning season than smaller males (Spearman's rank correlation test: $\rho = -.40$, $p = .02$).

Multiple males shared the same nest as co-breeders (mean number of males each nest = 3, range = 1–5), and they constructed an average of 2 nests during the spawning season (range = 1–5). The linear mixed-effects model showed that larger males invested more reproductive effort than smaller males on given nests (overall mean effect of body size across nests = 0.44, $SE = .15$, $p = .01$; Figure 2). Nest size increased when more males constructed together (mean effect = 0.62, $SE = .21$, $p = .01$; Figure 3), whereas nest size did not increase with male body size (mean effect = 0.05, $SE = 0.21$, $p = .80$).

Similar to male-size-mediated reproductive effort, the hierarchical regression model showed that larger males had higher reproductive success after accounting for variation among nests (posterior mean $\beta = .65$, 95% CI = 0.23–1.08). Male reproductive success also increased with nest size ($\gamma_1 = 3.07$, 95% CI = 1.14–5.08). The cumulative random effects across nests ($\sum_j w_{ij} \alpha_j$) in Equation (1) increased significantly with the cumulative sum of nest size where males were detected (Pearson's correlation test: $r = .76$, $p < .001$; Figure 4a), but did not depend on the number of nests that males constructed (Pearson $r = .25$, $p = .15$; Figure 4b). Therefore, nest size was a determinant of male reproductive success, while simply constructing more nests did not lead to more successful reproduction. Finally, the cumulative nest random effects ($\sum_j w_{ij} \alpha_j$) did not depend on male body size (Pearson correlation test: $r = -.15$, $p < .40$; Figure 5), providing further evidence that larger males did not construct larger nests. Taken together, our results suggested that building larger nests with other males increased reproductive success, but once on a given nest, larger males spent more reproductive

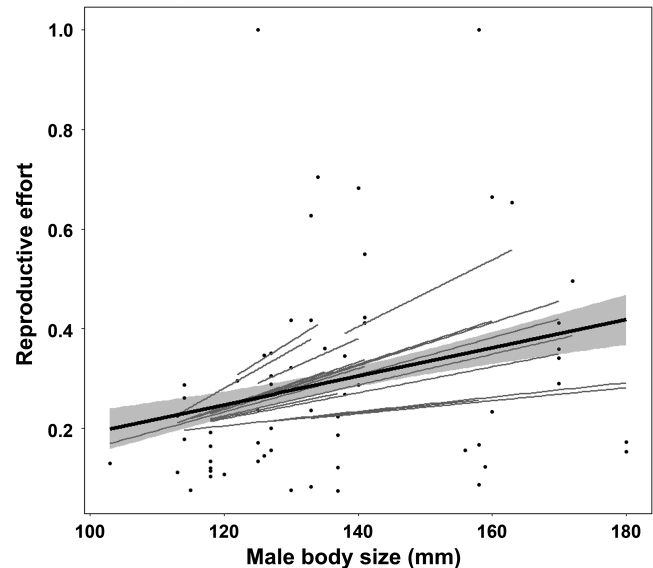


FIGURE 2 Predicted relationships between male body size and relative reproductive effort (i.e., proportion of detections by passive integrated transponder (PIT) antennas on each nest). The black line indicates the mean overall response across nests and gray shading shows 95% confidence interval. The gray lines represent a nest-specific pattern

effort than smaller males and consequently were more successful reproductively.

4 | DISCUSSION

Our findings supported two of the three hypothesized behavioral mechanisms to increase reproductive success of co-breeding males which may build multiple nests in a single spawning season. Larger males invested more effort on a given nest than smaller males, and consequently were more reproductively successful. Thus, a within-nest measure of effort was a correlate of male reproductive success. In addition, male reproductive success varied by nest. Reproductive success was higher for males who constructed larger nests together with more co-breeding males but constructing more nests did not increase reproductive success. These results showed that reproductive success of co-breeding males increased due to multiple behavioral mechanisms.

Positive relationships between male body size and reproductive success are commonly reported in co-breeding species (Friedl & Klump, 2000; Theuerkauf et al., 2009) and others (Barber, 2013; Bose et al., 2018; Lehtonen et al., 2007). Agonistic behavioral interactions occur on nests and their outcomes depend on body size (Hellmann et al., 2020; Soler et al., 1998; Taborsky, 1994). Larger males of bluehead chub at times displayed aggressive behavior toward each other and toward smaller males occupying the same nest in this study (S. Kim, *pers. obs.*) and others (Maurakis et al., 1991; Sabaj et al., 2000; Wallin, 1989). A few males (2–5) occupied a given nest and guarded their own spawning pits by tolerating each other as

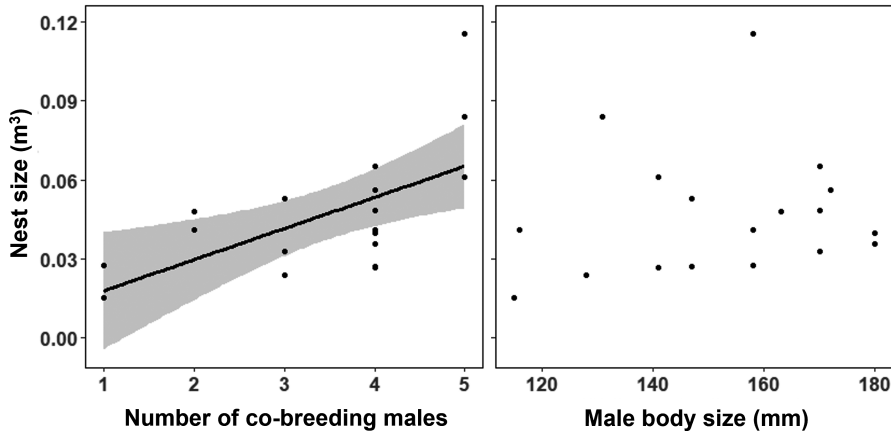


FIGURE 3 Effects of the number and mean body size of males on nest size ($n = 18$). Black line indicates mean response, and gray shading shows 95% confidence interval

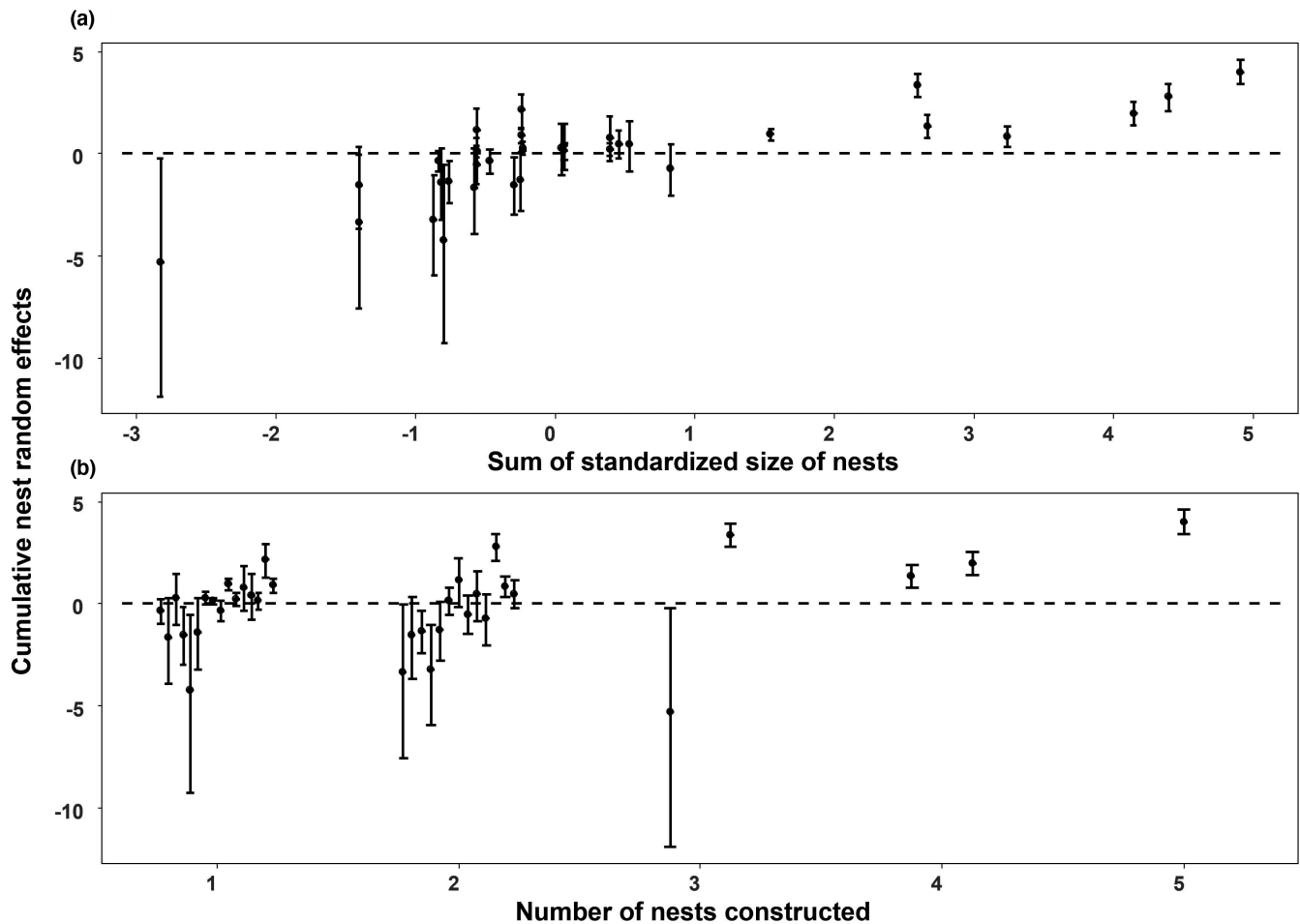
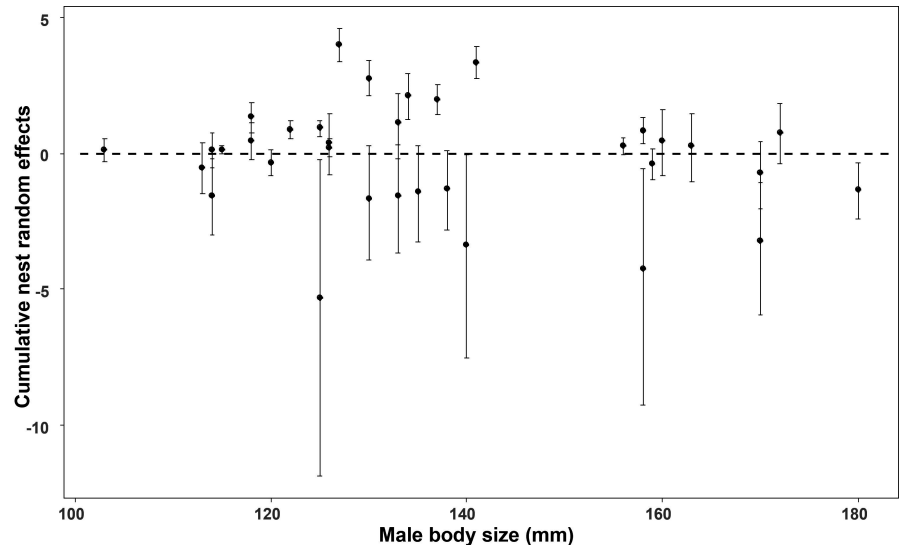


FIGURE 4 The relationship between cumulative nest random effects ($\sum_j w_{ij} \alpha_j$), and (a) sum of standardized size of nests and (b) number of nests each male constructed. Bars and dots represent 95% posterior credible intervals and mean values, respectively. Estimates with 95% credible intervals that do not bound zero (dashed line) are considered statistically significant

co-breeders (Kim, Peoples, et al., 2020; Sabaj et al., 2000), although more males were often detected by PIT antennas briefly. Sneaking behavior on nests is not known in bluehead chub (Kim, Pregler, et al., 2020). Accordingly, larger males did not solely occupy nests, resulting in positively size-dependent patterns of reproductive effort and success on single nests. Bluehead chub are short-lived (<3–4 years) but iteroparous with 2–3 potential years of spawning (Lachner, 1952;

Marcy et al., 2005). Given the few annual opportunities to reproduce, we speculate that larger and presumably older males maximize reproductive effort in the current breeding season, but smaller, younger males balance current reproductive effort and its effects on subsequent survival and reproductive success while subject to the current constraint imposed by larger males due to their agonistic behavior (Gross, 2005; Taborsky, 2001).

FIGURE 5 Relationship between male body size and cumulative nest random effects ($\sum_j w_{ij} \alpha_j$) on reproductive success of 34 males. Bars and dots represent 95% posterior credible intervals and mean values, respectively. Estimates with 95% credible intervals that do not bound zero (dashed line) are considered statistically significant. The mean posterior cumulative nest random effects did not depend on male body size (Pearson's correlation test: $r = -.15$, $p = .40$)



In a previous study, we reported that body size of males did not affect their reproductive success in the study stream (Kim, Pregler, et al., 2020). Reproductive success varied most among nests due to their behavior to build larger nests with more males in this study; thus, the effect of behavior was comparable to or more important than that of body size. The inconsistent importance of body size between the earlier and current studies is likely due to three reasons. First, the current study used a subset of data reported by Kim, Pregler, et al. (2020) by focusing on males detected by PIT antennas at the nests. A total of 64 males were marked with PIT tags between January and June 2017, but only 34 of them were detected on the 18 nests in the current study. We cannot know with certainty the fate of the males not detected by the antennas, whether it is mortality or emigration. It is plausible that body size differently affects reproductive success of males once they are spawning on nests vs. survival during the few months leading up to breeding. Second, the current analysis explicitly accounted for variation in male reproductive success among nests. The male body size effect on reproductive success is indeed not readily discernible on a biplot (Figure 5). By incorporating that male reproductive success varied among nests, our current analysis showed that male body size mattered relative to the size of other males on a given nest. Furthermore, our current approach assumed that reproductive success among males on the same nest is partitioned based on their relative effort measured by the time spent on the nest by each male. Third, larger males were detected earlier in the spawning season than smaller males, but Kim, Pregler, et al. (2020) did not incorporate this seasonal pattern. By incorporating the nest random effect in the hierarchical model, the current analysis was able to account for the seasonal pattern indirectly. Overall, the current result updates our previous knowledge and shows that male body size is indeed a predictor of reproductive success among males occupying the same nest.

Perhaps not surprisingly for a nest-building species, co-breeding with more males led to larger nests and higher reproductive success. It is noteworthy that the 5 most reproductively successful males (number of offspring: range = 13–33) spawned on larger nests

(range = 0.027–0.116 m³) than others (range = 0.015–0.084 m³). Nest size influences mate choice and reproductive success in many nest-building species (Barber, 2013; Bose et al., 2018; Soler et al., 1998). Likewise, larger nests attracted more female bluehead chub so that males could increase encounters with their potential mates (Kim, Pregler, et al., 2020). Larger nests were also visited and used for spawning by larger groups of yellowfin shiner in streams nearby, which would dilute predation effects on bluehead chub eggs (Kim, Peoples, et al., 2020; Silknetter et al., 2019). Because constructing more nests did not increase male reproductive success in the current study, our data indicated that males could best increase reproductive success by constructing larger nests and spent more effort on those nests.

Our data did not support one hypothesis that constructing more nests over the spawning season would increase male reproductive success. We considered that this behavior would function as a bet-hedging strategy in a stochastic environment where high flows due to precipitation mobilize substrates that destroy nests based on our previous studies (Kim & Kanno, 2020; Kim, Peoples, et al., 2020). In the current study, precipitation was recorded frequently prior to the spawning season but was recorded less frequently afterward (Appendix S2). Perhaps a bet-hedging strategy might be more effective in a less hydrologically stable condition. This idea could be tested in a longer-term study with contrasting summer precipitation regimes (i.e., dry vs. wet summers) or in a design that incorporates space-for-time substitution (i.e., free-flowing vs. flow-regulated rivers). A longer-term study to keep track of the same individuals in multiple years would also show whether nesting behaviors, such as number of nests to build in a single spawning season and number of males to build nests with, would change through ontogeny.

We were challenged to analyze this complex dataset because the nest from which YOY originated could not be identified via the genetic assignment method, given that males were commonly recorded on multiple nests by the PIT antennas. An alternative approach would have been to collect eggs from nests (Silknetter et al., 2019). This requires lethal sampling and is logistically

difficult because eggs of nest associates outnumber greatly those of bluehead chub on nests (Cashner & Bart, 2010; Silknetter et al., 2019). We also experimentally tested the effect of male body size on reproductive success, but with equivocal results (Kim, 2019), reflecting the difficulties of controlling body size and number of males in a manipulative experiment in the wild. Sampling YOY in the current study integrates nest origin and survival in the subsequent few weeks, an approach suitable for measuring animal reproductive success (Hunt & Hodgson, 2010). Ultimately, we devised a model-based solution by accounting explicitly for memberships of males to nests and reproductive effort of each male relative to others on each nest.

Male bluehead chub exhibited a unique co-breeding behavior to examine the relationship between allocation of efforts and reproductive success in nest-building species. Co-breeding behaviors are often observed in some animal taxa, particularly in fishes. For example, males of darters (Percidae) and suckers (Catostomidae) share spawning grounds and display mutual tolerance for reproduction (Díaz-Muñoz et al., 2014; Stiver et al., 2013). However, reproductive effort of those fishes is relatively less intensive than that of nest-building species, who invest much more effort in nest construction by collecting nesting resources. Thus, males of nest-building species are likely to occupy nests individually or reproduce as a group by displaying a social hierarchy, including breeders (dominant males) and helpers (subordinates) (Balshine et al., 2001; Bose et al., 2018; Hellmann et al., 2020; Sherley, 1990; Theuerkauf et al., 2009). By contrast, male bluehead chub co-breed on the same nest, while males provide intensive parental care by moving thousands of pebbles (Kim, Peoples, et al., 2020; Sabaj et al., 2000; Wallin, 1989). As such, reproductive success was likely determined by males' efforts on the quality nest but not social relationships. These unique behaviors enabled us to examine how allocation of reproductive efforts within and among nests affected reproductive success.

In conclusion, effort within nests was a stronger determinant of male reproductive success than effort among nests. This within-nest effort consisted of larger males spending more effort than smaller males often accompanied by agonistic interactions, and more males building larger nests together. Coexistence of these two seemingly different mechanisms, aggression and cooperation, is not a shared behavior among species in genus *Nocomis*, and indeed this combination of male reproductive behaviors is rare among fishes (Taborsky, 1994, 2009). Our study in this unique system demonstrated that aggression and cooperation shaped male reproductive success. This study provides a novel empirical insight into the evolution of mating systems because previous studies predominantly examined only aggression or cooperation (Díaz-Muñoz et al., 2014; Gross, 1996; Hellmann et al., 2020; Soler et al., 1998).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

SK and YK conceived the study. SK collected the field data. TLD provided oversight on the collection of genetic data and laboratory analysis. MBH, SK, and YK conducted the statistical analysis. YK and SK wrote the manuscript. SK revised the manuscript based on reviewer comments. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENT

The datasets used in this study are available from the corresponding author on reasonable request.

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