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Social Environment Influences the Temporal Dynamics of Sneak-Spawning in a Fish with Alternative Reproductive Tactics

Matthew C. Kustra,[1,*] Kelly A. Stiver,[2] Susan Marsh-Rollo,[1,3] Jennifer K. Hellmann,[4] and Suzanne H. Alonzo[1,5]

1. Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, California 95064; 2. Psychology Department, Southern Connecticut State University, New Haven, Connecticut 06515; 3. Department of Psychology, Neuroscience and Behaviour, McMaster University, Hamilton, Ontario L8S 4K1, Canada; 4. Department of Evolution, Ecology, and Behavior, School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801; and Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, Ohio 43210; 5. Institute of Marine Sciences, University of California, Santa Cruz, California 95064

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ABSTRACT: Several predictions of sperm competition theory are not well supported empirically. One potential reason is that most current theory and empirical research ignore how the social environment influence the temporal dynamics of mating. We propose that understanding these dynamics is key to understanding sexual selection and improving the predictive power of theory. To demonstrate the importance of these dynamics, we quantify how males' social role, interactions among males, and current social environment influence the timing of mating in *Symphodus ocellatus*, a species with three alternative male reproductive tactics. Nesting males spawn synchronously with females; sneakers and satellites sneak-spawn with some time delay. Satellites also cooperate with nesting males. We found that satellites have shorter sneak-spawning delays than sneakers, a benefit of their cooperation with nesting males. Sneak-spawning delays decreased with increasing nest activity for sneakers but not for satellites, suggesting that sneakers may benefit from increased sperm competition intensity. Current sperm competition models ignore this potential benefit, which may be why the prediction that males should decrease investment when sperm competition involves more than two males is not well supported. Our study provides insight into mechanisms that drive variation in the timing of spawning, which could explain mismatches between theoretical and empirical results.

Keywords: alternative mating tactics, sperm competition, cooperation, sneak-spawning, sexual selection, reproductive competition.

Introduction

A central goal of biology is to develop theory that can explain and predict empirical patterns. When empirical observations frequently deviate from theoretical predictions, it is important to ask—what is current theory missing? Despite extensive theory and empirical research (Parker 1970; Parker and Pizzari 2010; Lüpold and Pitnick 2018; Lüpold et al. 2020; Kahrl et al. 2021), several predictions of sperm competition theory are not well supported empirically. One potential reason could be that most models ignore active roles of females (Parker and Pizzari 2010; but see Ball and Parker 2003; Alonzo and Pizzari 2010; Requena and Alonzo 2014; Bocedi and Reid 2016). However, we propose that another often-overlooked aspect of postmating intrasexual selection theory is that the absolute timing of mating can influence selection on ejaculate traits and fertilization success. This is true for both species with internal fertilization (i.e., differences in time between copulations of different males with the same female; Pizzari et al. 2008; Manier et al. 2010, 2013; Smith 2012; Carleial et al. 2020) and external fertilization (i.e., differences in time between sperm release of different males and the same set of eggs; Stoltz and Neff 2006*a*, 2006*b*; Yeates et al. 2007; Egeland et al. 2015; Ota and Kohda 2015; Taborsky et al. 2018; Fitzpatrick 2020). Thus, success in postmating intrasexual selection is a product of at least two steps: (1) selection for traits and behaviors that decrease the time delay between multiple male mating events and (2) selection on ejaculate traits that favor fertilization. While much research has focused on ejaculate traits (step 2), selection on these traits is irrelevant unless

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[*] Corresponding author; email: mkustra@ucsc.edu.

ORCIDs: Kustra, https://orcid.org/0000-0002-1144-7030; Stiver, https://orcid.org/0000-0002-0650-8281; Marsh-Rollo, https://orcid.org/0000-0002-8488-3618; Hellmann, https://orcid.org/0000-0003-0479-3960; Alonzo, https://orcid.org/0000-0001-7757-0528.

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the male can gain timely access to advantageous fertilization opportunities (step 1). For example, sperm velocity is unimportant if a male mates after the female's eggs have already been fertilized by males able to mate more quickly. Yet little is known about how the immediate social environment influences the absolute timing of mating (step 1). We argue that this limits our ability to develop postmating sexual selection theory that accurately predicts empirical patterns.

For example, sperm competition theory generally predicts that males should allocate more energy to an ejaculate during mating when the immediate risk of sperm competition (defined as the probability a female mates with more than one male) is greater but decrease allocation to the ejaculate as the immediate intensity of sperm competition (defined as the number of males mating with the same female) increases (Parker and Pizzari 2010). Meta-analyses have shown that predictions for plastic ejaculate allocation in response to the immediate risk of sperm competition are generally well supported (Immler et al. 2011; Kelly and Jennions 2011). However, predictions about ejaculate allocation in response to immediate intensity of sperm competition are not well supported by the available data (Kelly and Jennions 2011). This lack of support could result from most sperm competition theory ignoring how the social environment (e.g., number of competing males) affects the absolute timing or positioning of gamete release, which affects fertilization success in many species (Parker and Pizzari 2010). Although previous empirical and theoretical work has focused on behaviors such as mate guarding (Parker 2020), studies tend to focus on (1) how it decreases the risk/intensity of sperm competition and (2) on relative mate-ordering affects. Consequently, there are few empirical studies quantifying the absolute timing of mating and how the social environment and behavioral interactions influence these temporal dynamics (Stoltz and Neff 2006*a*; Brattli et al. 2018). Thus, empirical work on these dynamics is crucial to help inform the development of future theory.

Systems with alternative reproductive tactics (ARTs)—discontinuous variation in behavior, physiology, or morphology within a sex to achieve reproductive success in alternative ways (Gross 1996; Taborsky et al. 2008)—have been used extensively to test predictions of sperm competition theory and are therefore powerful systems for studying the effect of temporal dynamics in mating events. Male ARTs are common across taxa and often involve a dominant territory-holding tactic and a sneaker tactic that exploits the dominant male's mating opportunities (Gross 1996; Taborsky et al. 2008). As sneakers are almost always in sperm competition, postmating sexual selection is strong and the intensity of sperm competition is often high. Sneakers are also often at a temporal disadvantage, which likely influences selection on ejaculate traits and on traits that influence the timing and position of mating (Taborsky et al. 2018; Kustra and Alonzo 2020). Thus, the temporal dynamics of sneaking (mating with a female after a dominant male has mated) will also be important in the evolution and maintenance of ARTs. However, few studies have quantified observed variation in sneak-spawning times, and even fewer have determined how factors, such as the social environment, influence the spatiotemporal dynamics of mating (Stoltz and Neff 2006*a*; Ota et al. 2010; Sørum et al. 2011; Ota and Kohda 2015; Brattli et al. 2018; Ota 2019). For example, if many sneakers attempt to sneak simultaneously, they may interfere with each other. Their sneak-spawning delays—the difference between the time a dominant male and a subordinate male mate with a female—could increase, likely lowering the probability of fertilization. Alternatively, spawning situations with many males involved may prevent a dominant male from effectively defending against sneakers, resulting in shorter sneak-spawning delays for sneaker males. We argue that understanding and quantifying how social interactions and the social environment affect the temporal dynamics of mating is essential for developing sperm competition theory with greater predictive power.

Here, we investigate how the social interactions between males of different tactics and the social environment influence the timing of mating. We study this in the ocellated wrasse (*Symphodus ocellatus*), a species with three ARTs: a large dominant nesting male, an intermediate-sized satellite male, and a small sneaker male (Lejeune 1984; Taborsky et al. 1987; Alonzo and Warner 2000*b*). These ARTs allow us to look at how the social role and social environment affect the timing of mating. The male tactics are likely condition dependent based on juvenile growth rates (Alonzo et al. 2000). Males with fast growth will be satellite males in their first reproductive season and nesting males in their second and final reproductive season. Males with slow growth will be sneaker males in their first year and then satellite males in their second and final reproductive year (Alonzo et al. 2000). Sneaker males adopt a purely parasitic ART; satellites, on the other hand, are partially cooperative with nesting males. Satellite males ally sequentially with various nesting males across the reproductive season and expend energy aiding the nesting male by chasing away sneakers and courting females (Taborsky et al. 1987). However, both satellite males and sneaker males primarily achieve reproductive success through sneak-spawning (Stiver and Alonzo 2013). Satellite males are socially dominant to sneaker males and are allowed closer to the nest by the nesting male. This may increase satellite male reproductive success via shorter sneak-spawning delays relative to sneaker males (Taborsky et al. 1987; Stiver and Alonzo 2013). However, the benefits of being a cooperative satellite tactic over a pure sneaker tactic remain an open question.

We analyzed behavioral observations across multiple years to better understand the temporal dynamics of mating in this system. We first tested whether satellite males have shorter sneak-spawning delays than sneakers and whether this differed depending on the immediate sneaking situation ("single sneaker or satellite," "single sneaker and satellite," or "multiple sneakers and a satellite"). We predicted that satellite males would have shorter sneak-spawning delays than sneaker males because they are allowed closer to the nest by the nesting male (as hypothesized in Taborsky et al. 1987). Next, we tested how (1) nest activity (e.g., number of female visits, number of spawning events) and (2) interactions between males (e.g., nesting male aggression to satellite males and sneakers) influence sneak-spawning delays. We predicted that sneak-spawning delays would increase with nest activity because more competition could cause interference among males. However, the opposite effect may happen if nesting males are distracted at busy nests, allowing sneakers to get closer and have shorter sneak-spawning delays. Finally, we predicted that nests with more aggressive nesting males would have longer sneak-spawning delays, as both sneaker and satellite males may be forced farther away from the nest.

Methods

Live Observations

This research was conducted at the University of Liege Marine Station (STARESO) near Calvi, Corsica, France (42.5806°N, 8.7243°E), from mid-May to mid-June (the breeding season of *Symphodus ocellatus*) in 2014 and 2016–2019. We performed 10-min live behavioral observations using SCUBA on haphazardly selected *S. ocellatus* nests. We focused on nests with a spawning nesting male, at least two sneaker males, and a satellite male present for us to record sneaks from multiple males. During the observations, we quantified social interactions between male ARTs: the number of aggressive behaviors from the nesting male to sneaker males, the nesting male to the satellite male, and the satellite male to sneaker males. We also recorded the number of submissive behaviors of the satellite male to the nesting male. For a more in-depth description of these behaviors, see Stiver and Alonzo (2013). Every minute, we counted the number of sneakers within ~1 m of the nest (estimated visually) that were also oriented toward the nest or interacting with other individuals at the nest. To measure sneaker presence, we averaged the number of sneakers counted per minute in the 10-min observations. We also recorded the total number of female visits, the total number of females that spawned, the total number of spawns that occurred, the number of sneaks from sneakers, and the number of sneaks from satellite males. We concurrently recorded a video of these live observations to quantify sneak-spawning delays—the difference between the time when the nesting male spawned and the time when the subordinate male(s) spawned.

Sneak-Spawning Delay Data Collection

To quantify the sneak-spawning delay of sneaker and satellite males, we analyzed the 10-min nest focal videos shot at 30 fps that were synchronized with the live observations described above using QuickTime 7 (2014 and 2016) and BORIS 7.11.1 (2017–2019; Friard and Gamba 2016). For each sneak-spawning event (e.g., fig. 1), we recorded the time to the millisecond when the nesting male spawned with the female and the time when either a sneaker or a satellite male completely entered the nest to sneak-spawn (fig. 1; video S1). We calculated the sneak-spawning delay by subtracting these two times. To match observations recorded from QuickTime 7, which rounded observations to the nearest millisecond, we rounded all BORIS sneak-spawning delay observations to the nearest millisecond. We also recorded the number of males that sneaked in each sneak-spawning event and what type of males sneaked (i.e., sneakers and/or a satellite). To focus on natural spawning behaviors, we included only videos for which an experimental manipulation did not occur. We excluded videos that did not have at least one sneaker and one satellite sneak-spawning event because we wanted to compare the sneak-spawning delays of both male types. We also excluded nests with more than one satellite (typically, nests have only one satellite). We excluded any sneak-spawning delay observations if the video was too blurry to make the sneak-spawning delay difficult to measure accurately. This resulted in the following final nest sample sizes ($n = 34$): for 2014, $n = 10$ nests; for 2016, $n = 9$ nests; for 2017, $n = 4$ nests; for 2018, $n = 6$ nests; and for 2019, $n = 5$ nests.

Statistical Analyses

We first wanted to test how sneaker and satellite males differed in sneak-spawning delays and whether that was affected by the social makeup of specific sneaking events. We categorized all sneaking events into three sneaking categories: the single sneaker or satellite category (a sneaker or the satellite sneak-spawned alone; $n = 34$ satellite observations; $n = 79$ sneaker observations), the single sneaker and satellite category (one sneaker male and the satellite male sneak-spawned at the same sneaking event; $n = 46$ satellite sneaks; $n = 46$ sneaker sneaks), and the multiple

Figure 1: *Symphodus ocellatus* nest with all three male tactics and female. The nesting male (shown in his nest) builds nests, courts females, and provides parental care. Females have a strong mating preference for nesting males and spawn in synchrony with them. Satellite males aid nesting males by chasing away sneakers and courting females, but they also perform sneak-spawns. As shown above, satellite males are allowed closer to the nest than sneaker males. Photograph by S. Marsh-Rollo.

sneakers and satellite category (multiple sneaker males and the satellite male sneak-spawned at the same sneaking event; $n = 31$ satellite sneaks; $n = 80$ sneaker sneaks). We used this grouping instead of using the number of parasitic males because of much lower sample sizes after more than three males (fig. S1). If a spawn fell into the multiple sneakers and satellite category, we used all sneak-spawning delay times in the analysis. We handled the nonindependence of these observations with random effects (described below). We excluded sneaking events with multiple sneakers but no satellite male because there would be no satellite male observations in this category, and the goal of this analysis was to look at differences between the tactics. This resulted in the total sample sizes of $n = 111$ satellite sneaks and $n = 205$ sneaker sneaks. To ensure that any result was not simply due to the number of males as opposed to the presence of a satellite, we performed an analogous analysis with sneaker-only observations ($n = 191$ sneaker sneaks).

We analyzed the data using a hierarchical Bayesian generalized linear regression with a gamma family and log link (i.e., a generalized linear mixed model) using the brms package (Hobbs and Hooten 2015; Bürkner 2018). We chose a gamma family because the response variable, sneak-spawning delay, is strictly positive and skewed. The fixed effects of this test were male type (satellite or sneaker), the category of sneaking event, and their interaction. The random effects were sneaking event nested within the fish nest of an observation nested within year. For this model, we used uninformative priors (Hobbs and Hooten 2015). Specifically, we used normal (mean = 0, SD = 1,000) for the intercept and fixed effects, inverse gamma ($\alpha = 0.001$, $\beta = 0.001$) for random effect variations, and gamma ($\alpha = 0.01$, $\beta = 0.01$) for the gamma shape parameter. We ran models with four chains with 5,000 iterations per chain, discarding the first 1,000 as burn-in (Hobbs and Hooten 2015). After we ran the models, we extracted posterior sample draws to estimate differences in sneak-spawning delays between satellite and sneaker males in the different sneaking categories. For all model parameters, we then computed the evidence ratio—the ratio of the posterior probability that shared the same sign as the estimate to the posterior probability with the opposite sign (Bürkner 2018). For example, suppose a model parameter estimated effect was positive. In that case, an evidence ratio of 10 indicates that the parameter is 10 times more likely to have a positive effect than a negative effect. To avoid null-hypothesis significance testing with arbitrary cutoffs (Muff et al. 2022), we interpret our results considering the evidence ratio, estimated effect sizes, and 95% credible intervals (CIs).

We next wanted to test how social interactions between males and the overall nest activity influenced the sneak-spawning delay of sneaker and satellite males. Because many of the behaviors and measurements of nest activity were highly correlated, we performed a principal component analysis (PCA) on all variables collected during live observations described in the first section ($n = 34$ nests). We then performed randomization tests to (1) make sure the data were structured in a way that a PCA would be meaningful and (2) select principal components (PCs) that were biologically meaningful (e.g., variance explained was not due to random chance; Björklund 2019). See the "Supplemental methods" section of the supplemental PDF for more details (figs. S2–S4).

We used two PCs in our analysis. PC1 was representative of variables related to nest activity (fig. S3). Specifically, it was positively loaded by the total number of spawns (17.258% contribution), sneaks (16.987% contribution), sneaks performed by sneakers (16.120% contribution), female spawns (14.866% contribution), female visits (13.346% contribution), and sneaks performed by satellite males (12.903% contribution). PC2 was representative of variables that explained male-male interactions (fig. S3). Specifically, it was positively loaded by the number of satellite male to sneaker aggressions (31.142% contribution), satellite to nesting male submissions (21.899% contribution), and average sneaker presence (23.883% contribution).

We then performed a hierarchical Bayesian generalized linear model with a gamma distribution predicting sneak-spawning delay with fixed effects of male type, nest activity (PC1), male interactions (PC2), and the interaction of these effects with male type ($n = 111$ satellite sneaks; $n = 317$ sneaker sneaks). We included random effects of spawning events nested within fish nest of an observation nested within year to account for the nonindependence of observations. We used the same priors and model methodology (e.g., number of chains and iterations) described in the first analysis.

All statistical models reached convergence without error, and all parameters had $\hat{r} \approx 1$, indicating that the separate chains converged. Additionally, posterior predictive checks indicated that all statistical models reasonably fit the data. To ensure the choice of priors did not substantially influence our results, we also ran all models using weakly informative regularizing priors for parameter effects, normal (mean = 0, SD = 3). Using these weakly regularizing priors yielded qualitatively and quantitatively similar results (tables S1, S2). We also included a full model with both nest-level effects and spawning situations to ensure that they did not explain each other (e.g., the effect of sneaking situations might be explained by differences in nest activity). This generally resulted in qualitatively and quantitatively similar results (unless noted otherwise) as when the effects were analyzed separately (table S3). We chose to analyze them separately so we would not have to exclude the situations when there were spawning events with multiple sneakers and no satellite male to test nest-level effects. We conducted all analyses and made all figures in R (ver. 4.1.2; R Core Team 2018) using the tidyverse suite of packages (Wickham et al. 2019). We give an in-depth breakdown of sample sizes across years and male types in table S5.

Results

How Much Do Satellite and Sneaker Males Differ in Sneak-Spawning Delays?

We found strong support (evidence ratio was large and CIs did not overlap zero) that satellite males had, on average, shorter sneak-spawning delays than sneaker males across all sneaking situations (satellite sneak-spawning delay − sneaker sneak-spawning delay < 0; fig. 2*B*; table 1).[1] This was true when a single sneaker or satellite sneak-spawned alone (estimated median difference: −0.0959 s; 95% CI: −0.1627 to −0.0286; evidence ratio: 89.3955), when a single sneaker and satellite sneak-spawned at the same event (estimated median difference: −0.1801 s; 95% CI: −0.2549 to −0.1103; evidence ratio: ∞),[2] and when multiple sneakers and satellite sneak-spawned at the same event (estimated median difference: −0.0998 s; 95% CI: −0.1554 to −0.0439; evidence ratio: 389.2439). We found that satellite males and sneakers in the multiple sneakers and satellite situation generally had shorter sneak-spawning delays than other sneaking situations (table 1; fig. 2*A*). We also found that there was likely an interaction between male type and when a single sneaker and satellite sneak-spawned (table 1). Specifically, sneaker males were slower with a satellite male present (single sneaker and satellite category) compared with alone (single sneaker or satellite category; median difference: 0.0434 s; 95% CI: −0.0312 to 0.122; evidence ratio: 4.6919) or with many sneakers and a satellite male (multiple sneakers and satellite category; median difference 0.1014 s; 95% CI: 0.0291 to 0.1779; evidence ratio: 99.6289). The general pattern for

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[1] Table 1 gives estimations of specific model effects, while estimates and CIs in the text are from contrasts extracted from the model.

[2] All posterior samples indicated that satellite males had shorter sneak-spawning delays than sneaker males.

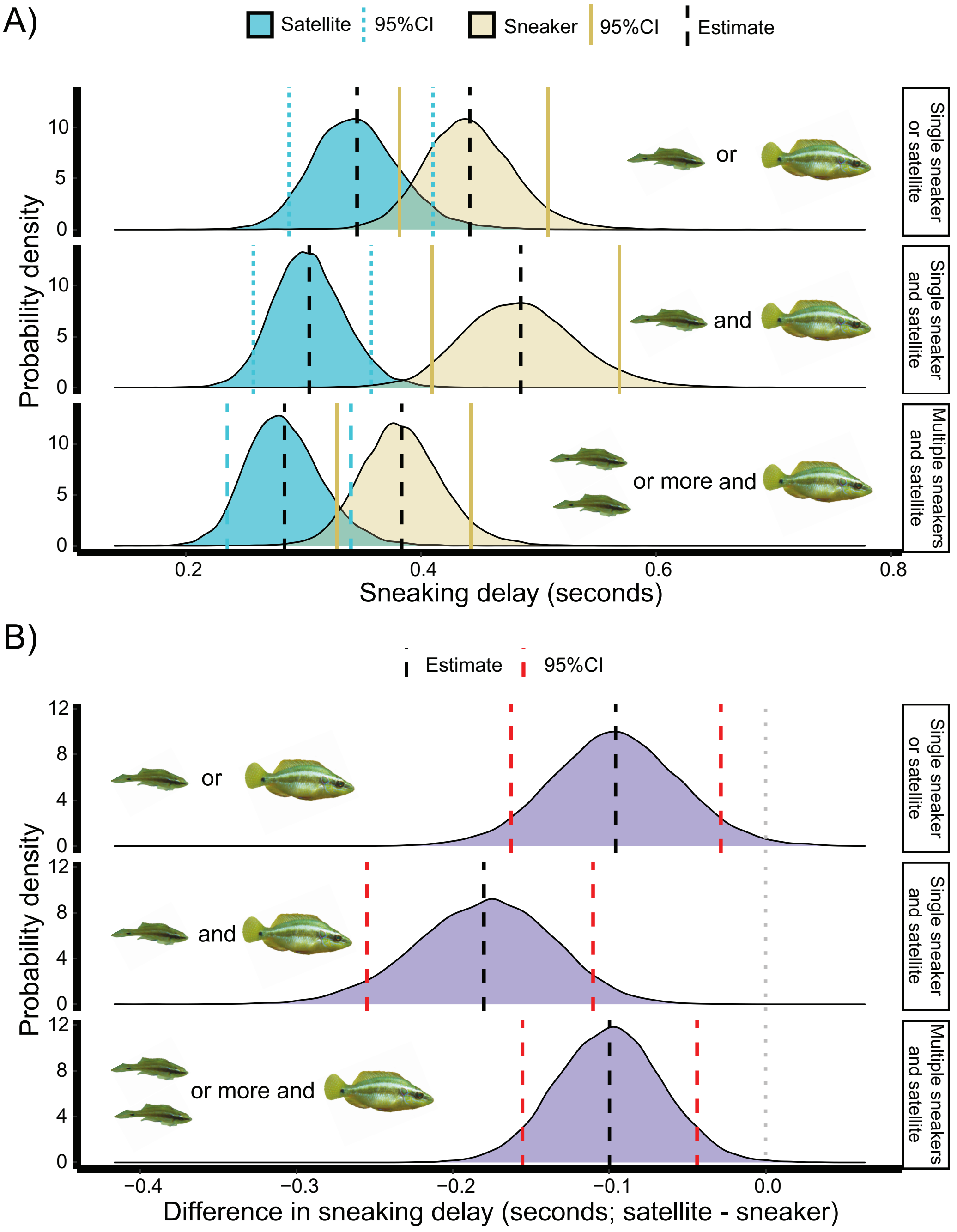

Figure 2: Male tactic and spawning scenario interact to influence the temporal dynamics of mating. Satellite males, on average, have a shorter sneak-spawning delay than sneaker males, with this effect being strongest during paired spawns. *A*, Posterior probability distributions of estimated average sneak-spawning delay for satellite (blue) and sneaker (gold) males when sneaks were performed with the single sneaker or satellite scenario, the single sneaker and satellite scenario, or the multiple sneakers and satellite scenario. Black dashed lines indicate medians, and colored dashed lines indicate 95% credible intervals (CIs) for different male types. *B*, Posterior probability distributions of the hypotheses testing the difference in sneak-spawning delay between satellite males and sneaker males when sneaks were performed with different mating scenarios. Black dashed lines indicate medians, red dashed lines indicate 95% CIs, and the dotted gray line is at zero (i.e., no difference). Left of the dotted gray line (negative numbers) indicate that satellite males had shorter sneak-spawning delays; right of the dotted gray line, sneakers had faster sneak-spawning delays (positive numbers). Posterior distributions are all from the same model described in table 1. Images of sneaker and satellite male are from the photo in figure 1 by S. Marsh-Rollo.

Table 1: Results of fixed effects from a hierarchical Bayesian generalized linear model with a gamma distribution predicting sneak-spawning delay as a function of male type, sneaking situation, and their interaction

Model effect	Estimate	Evidence ratio	Error	95% CI	Tail effective sample size
Intercept	***−.8221***	∞[a]	***.0894***	***−.9953* to *−.6470***	***6,552***
sat	***−.2472***	***89.3955***	***.1064***	***−.4522* to *−.0359***	***9,577***
sn + sat	.0927	4.6919	.0995	−.1020 to .2883	11,458
Multiple sn + sat	*−.1411*	*17.5400*	*.0879*	*−.3131* to *.0323*	*11,504*
sat: sn + sat	*−.2174*	*12.8528*	*.1506*	*−.5145* to *.0780*	*10,325*
sat: multiple sn + sat	−.0568	1.8556	.1501	−.3515 to .2381	10,506

Note: The random effects for this model were spawning event nested within the fish nest of an observation nested within year. The shape parameter of this model was estimated to be 4.2030 with an error of 0.3808; Bayesian R^2 was estimated to be 0.2883 with an error of 0.0539. Boldfaced rows indicate estimates with 95% credible intervals (CIs) that did not overlap zero; italicized rows indicate an evidence ratio >10 (10 times more likely effect shares the same sign as estimate than not). Model effects are compared with the sneak-spawning delay of a sneaker male when it is sneaking alone. CI = credible interval; sat = satellite male; sn = sneaker male.

[a] All posterior samples were negative.

sneaker-only spawns with the same number of total males was similar, but effect sizes and evidence ratios were much smaller (table S4; fig. S5). Moreover, the CIs always overlapped zero, indicating that the difference between the single sneaker and satellite situation and the multiple sneakers and satellite situation was in part driven by the presence of the satellite male and not just the number of males present (table S4; fig. S5). In summary, satellite males typically have shorter sneak-spawning delays than sneaker males, and this difference was largest when a single sneaker male and satellite male compete in the same sneaking event. Furthermore, sneakers had shorter sneak-spawning delays when multiple sneakers and a satellite sneak in the same sneaking event compared with when a single sneaker and a satellite sneak.

How Do Nest Activity and Male Interactions Affect Sneak-Spawning Delays?

We found strong support (the evidence ratio was large, and CIs did not overlap zero) for an interaction between nest activity (PC1) and male type on sneak-spawning delay (fig. 3*A*; table 2). Specifically, sneaker male delays decreased with increasing nest activity, but satellite male delays were not influenced by nest activity (fig. 3*A*; table 2). We found

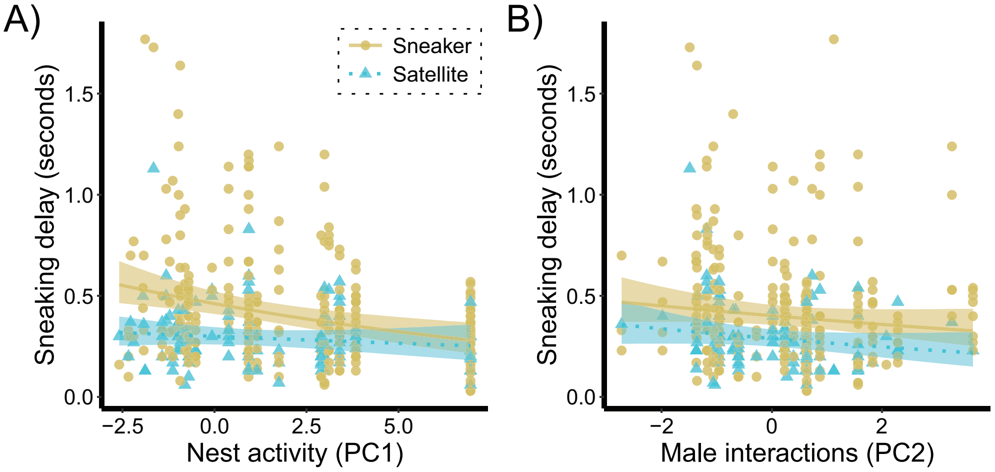

Figure 3: Social environment influences the temporal dynamics of mating. *A*, Sneak-spawning delay decreases with increasing nest activity (PC1) for sneakers but not satellite males. *B*, Sneak-spawning delay for both males decreases slightly with more male interactions (PC2). Lines are the median, and shading is the 95% credible intervals of the predicted sneaker (gold) or satellite (blue) sneak-spawning delay; points are raw data for sneakers (gold and circle) and satellites (blue and triangle). In *A*, high values of nest activity indicate nests with a high number of spawns, sneaks, and female visits. In *B*, high values of male interactions indicate a high average number of sneakers, a high number of satellite male to sneaker male aggressions, and a high number of satellite male to nesting male submissions. Predictions come from posterior samples of the model presented in table 2 conditioned on the mean of other model effects.

Table 2: Results of fixed effects from a hierarchical Bayesian generalized linear model with a gamma distribution predicting sneak-spawning delay as a function of male type, nest activity (PC1), male interactions (PC2), and their interaction with male type

Model effect	Estimate	Evidence ratio	Error	95% CI	Tail effective sample size
Intercept	***−.7610***	∞[a]	***.0620***	***−.8790*** to ***−.6377***	***9,925***
Satellite male	***−.4290***	∞[a]	***.0711***	***−.5664*** to ***−.2893***	***11,954***
Nest activity	***−.0714***	***2,665.667***	***.0202***	***−.1128*** to ***−.0314***	***8,550***
Male interactions	*−.0560*	*17.244*	*.0350*	*−.1246* to *.0146*	*10,890*
Satellite: nest activity	***.0476***	***53.4218***	***.0231***	***.0030*** to ***.0933***	***12,107***
Satellite: male interactions	−.0201	2.0075	.0478	−.1123 to .0746	11,925

Note: The random effects for this model were spawning event nested within the fish nest of an observation nested within year. The shape parameter of this model was estimated to be 4.1043 with an error of 0.3904; Bayesian R^2 was estimated to be 0.2709 with an error of 0.0581. Boldfaced rows indicate estimates with 95% credible intervals (CIs) did not overlap zero; italicized rows indicate an evidence ratio >10 (10 times more likely effect shares the same sign as estimate than not).

[a] All posterior samples were negative.

weak support that sneak-spawning delays for both male types decreased with more male interactions (PC2). Although the evidence ratio was >10, this effect was relatively small, and the 95% CIs overlapped zero (fig. 3*B*; table 2). Furthermore, including spawning situations in the same model almost halved the evidence ratio (table S3). In summary, sneakers had quicker sneak-spawns at busier nests, but male interactions had little influence on sneak-spawning times for both satellites and sneakers.

Discussion

In the context of postmating intrasexual selection, reproductive success depends on (1) traits and behaviors that decrease the time delay between multiple male mating events and (2) ejaculate traits that favor fertilization. Although there has been extensive research on ejaculate traits, few studies have quantified variation in mating time delays or determined how the social environment might explain this variation. Differences in the timing of mating will affect selection on ejaculate traits and therefore are likely critical to understanding the evolution and maintenance of ARTs. Here, we analyzed detailed behavioral observations from multiple years to better understand (1) how ARTs differ in sneak-spawning delays and how (2) nest activity and (3) social interactions influence the relative timing of male gamete release. We found that satellite males had shorter sneak-spawning delays than sneaker males, which was greatest when both a satellite and a single sneaker snuck at the same sneaking event. We also found that sneak-spawning delay decreased with increasing nest activity for sneaker males but not for satellite males. Given that small differences in sperm velocity have already been shown to affect paternity in this species (Alonzo et al. 2016) and that time delays in other fish species with external fertilization affect paternity (Stoltz and Neff 2006*b*; Yeates et al. 2007; Egeland et al. 2015), it is reasonable to assume that these differences in the timing of sperm release will likely affect paternity as well.

Regardless of the sneaking scenario, satellite males had shorter delays than sneaker males. This result is consistent with previous observations that satellite males are allowed closer to the nest than sneaker males (Taborsky et al. 1987; Stiver and Alonzo 2013). This difference was most prominent in sneaking situations with a single sneaker and satellite, primarily due to sneakers experiencing long delays when competing directly with a satellite male (fig. 2*A*). This could be because larger satellite males physically interfere with sneakers when sneaking together. Larger males also have an advantage when sneak-spawning in Masu salmon (*Oncorhynchus masou*; Koseki and Maekawa 2000) and dusky frillgoby (*Bathygobius fuscus*; Takegaki et al. 2012). Combined with these results, our finding suggests a need to reevaluate the general assumption that smaller size is favored when sneak-spawning (Ota et al. 2010). This result also indicates that faster sneak-spawning (and likely higher fertilization success) is one advantage of adopting a partially cooperative ART and may have enabled this third alternative male tactic to evolve.

We found that sneaker delay times were shortest when the spawning situation was busiest (the multiple sneakers and satellite situation) and nest activity was higher (greater number of spawns, sneaks, and female visits). These results imply that sneaker males, on average, benefit from sneaking when sperm competition is intense (more than two competitors). In these situations, sneaker males may be able to get closer to the nest or get to the nest faster, as nesting males may be preoccupied with courting females and fending off other sneakers. This dynamic is analogous to predator dilution effects. An additional and non-mutually-exclusive possibility is that sneaker males may adjust effort (swim faster or get closer to the nest) to get a completive edge over other parasitic males in these highly competitive scenarios. Spawning faster likely increases the probability of fertilization and thus could counteract some of the negative impacts of higher sperm competition intensity (i.e., paternity being shared with more males). However, current theoretical models have not incorporated how the social environment influences the dynamics of sperm competition. Incorporating these effects could alter current theoretical predictions that males should invest less when the intensity of sperm competition is high (Parker et al. 1996; Parker and Pizzari 2010) and could explain why these predictions are not generally well supported (Kelly and Jennions 2011).

Ignoring aspects of the relative timing and positioning of gamete release may also explain why sperm competition theory has failed to predict differences in ejaculate allocation among ARTs reliably. Sneakers are almost always in sperm competition. Postmating sexual selection is therefore important in systems with ARTs, and existing theory predicts that sneakers should invest more in ejaculate production than dominant (territory-holding) males (Parker 1990; Montgomerie and Fitzpatrick 2009; Kustra and Alonzo 2020; Dougherty et al. 2022). However, a recent review (Kustra and Alonzo 2020) and meta-analysis (Dougherty et al. 2022) found that this predicted pattern did not hold generally across species. We suggest that this could, in part, be due to selection on the timing of sperm release overriding selection on ejaculate traits, or the spatiotemporal dynamics of mating in these systems may explain how ejaculate investment varies between tactics. To improve our understanding of postmating sexual selection, we need more empirical work quantifying the temporal mating dynamics of ARTs. We also need to develop theory on ejaculate allocation in ARTs that (1) incorporate investment into nonejaculate components that influence the competitive weighting of ejaculates (e.g., the energy devoted to quicker sneaking) and (2) allow the number of sneakers in a mating to influence the competitive weighting of sneaker ejaculates. Incorporating these aspects may better align theoretical expectations with empirical realities.

Instead of overriding selection on ejaculate traits, the timing of fertilization could also change the relative importance of ejaculate components (Bakker et al. 2014; Egeland et al. 2015). For example, spawning at time delays decreased the relative importance of sperm number in the Arctic charr (*Salvelinus alpinus*), which may explain why subordinate males produce faster sperm (Egeland et al. 2015). Similarly, in the ocellated wrasse, sneaker males may compensate for sneak-spawning delays as they release approximately four times more sperm than satellite or nesting males (Alonzo and Warner 2000*a*). However, nesting males produce higher-quality ejaculates than sneaker males (Alonzo et al. 2016), and sperm production does not differ between male types (Alonzo et al. 2021). Sperm do not compete in a vacuum, and in many species females can have considerable influence on sperm competition dynamics (Zadmajid et al. 2019; Gasparini et al. 2020; Myers et al. 2020). For example, in this system ovarian fluid influences sperm competition dynamics by increasing the relative importance of sperm velocity and the speed at which sperm may fertilize the egg, further favoring nesting males (Alonzo et al. 2016). The total selective pressure acting on ejaculate traits is likely a combination of sperm competition, the female environment, and the temporal dynamics of mating. Developing new theory that considers all three aspects can help guide future work and provide testable predictions. Furthermore, future empirical work should explicitly test these interactions to help improve our understanding of the evolution of ejaculate traits and behavior.

There are a few key limitations to this study that may affect our interpretation of our results. First, in this system we do not currently know the extent to which these time delays may influence paternity. However, we do know that fertilization occurs rapidly in species with external fertilization, and small time delays can have huge influences in paternity in other systems (Stoltz and Neff 2006*a*; Yeates et al. 2007; Egeland et al. 2015). Furthermore, small differences in sperm velocity in this system (Alonzo et al. 2016) and others influence paternity. Second, our measure of male interactions (PC2) was from the entire 10-min observation period, which could have driven our finding of weak evidence for an effect of male interactions. It is quite plausible that the interactions between males that happen immediately before spawning events may have impacts on time delays and are more important than average rates of interactions. To improve our understanding of the temporal dynamics of mating and fertilization success, future empirical work in this system and others should analyze how sequences of behaviors/interactions influence time delays that in turn influence fertilization outcomes.

Most research on postmating sexual selection, both theoretical and empirical, has ignored how the social environment may influence the absolute timing of mating. Our study shows that the social environment can affect the fine-scale temporal dynamics of mating and highlights the potential for important feedbacks among the social environment, sperm competition, and the evolution of ARTs. Future work in other systems should explore how the social environment and interactions between competitors influence the temporal dynamics of mating and how time differences in mating interact with sperm competition and cryptic female choice. Furthermore, our results show that the social makeup of mating situations can influence the timing of mating. Incorporating such dynamics into future theory may better align theoretical predictions and empirical results.

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Statement of Authorship

M.C.K. and S.H.A. conceived of the study, and S.H.A. designed the protocols. All authors collected the data used in this article. M.C.K. performed the statistical analyses and drafted the manuscript. All authors critically revised the manuscript and approved publication of this article.

Data and Code Availability

Code and data needed to run the model, make figures, and perform statistical analyses have been deposited in the Dryad Digital Repository (https://doi.org/10.7291/D17698; Kustra et al. 2023).

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