

Strength of Sexual Selection and Sex Roles Vary between Social Groups in a Coral Reef Cardinalfish

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ABSTRACT: The strength and direction of sexual selection can vary among populations. However, spatial variability is rarely explored at the level of the social group. Here we investigate sexual selection and sex roles in the paternally mouthbrooding, socially monogamous, and site-attached pajama cardinalfish, *Sphaeramia nematoptera*. Females were larger and more aggressive and had a longer dorsal fin filament, indicating reversed sex roles. At the scale of social groups, we show that the Bateman gradient and reproductive variance depend on the sex ratio and size of groups. In small and medium-sized groups with balanced or male-biased sex ratios, Bateman gradients were steeper for females, whereas gradients were equally steep for both sexes in large groups or when the sex ratio was female biased. For both sexes, reproductive variance increased with group size and with a higher male-to-female sex ratio. In *S. nematoptera*, mating opportunities outside the socially monogamous pair appear to impact sexual selection. We conclude that strength and direction of sexual selection can be masked by social dynamics in group-living species when considering only population and large-scale demographic processes.

Keywords: sexual selection, reversed sex roles, Bateman gradients, reproductive variance, reproductive skew, sexual dimorphism.

Introduction

An increasing body of evidence suggests that the strength and direction of sexual selection in the wild can vary spatially among geographically distinct populations (McLain 1982; Forsgren et al. 1996; Mobley and Jones 2007, 2009) and

temporally within populations (Downhower et al. 1987; McLain 1992; Kasumovic et al. 2008; Wacker et al. 2014; Cunha et al. 2015; Oyama et al. 2020; but see Monroe et al. 2016). In many cases, predictable seasonal change in biotic variables influences the operational sex ratio (OSR; the ratio of males and females ready to mate; Emlen 1976; Emlen and Oring 1977), the adult sex ratio (ASR; the ratio of sexually mature males and females in the population; Kokko and Jennions 2008a), and/or the density of one or both sexes (McLain 1982, 1992; Jann et al. 2000; Kasumovic et al. 2008; de Jong et al. 2009). Sex roles may switch from male-male competition and female mate choice when the OSR or ASR is male biased to female-female competition and male mate choice when the OSR or ASR is female biased (Forsgren et al. 2004; Kappeler 2017; Amundsen 2018). Furthermore, within-sex competition may be influenced by population density (Emlen and Oring 1977; McLain 1992; Jann et al. 2000; Mills et al. 2003; Forsgren et al. 2004; Kokko and Rankin 2006; Kasumovic et al. 2008). However, these studies are based on population-wide estimates of variation. Reproductive individuals interact with only a fraction of the population, and it is at the local level that competition for breeding status or intrasexual competition occurs. Understanding how sex roles and sexual selection differ with spatial and temporal variability at the local level can advance our understanding of the role of local and social dynamics in the evolution of mating systems (Kasumovic et al. 2008; Kappeler 2017).

The strength and direction of sexual selection are influenced by differences in population size and structure at a scale that is smaller than that of an entire population,

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particularly in animals that show high site fidelity or that are group living (Kasumovic et al. 2008; Kappeler 2017). Animals with high site fidelity may be exposed to a varying social environment depending on the group or site they are attached to, and sexual selection can be variable and sex roles phenotypically plastic in response to this environment (Arnqvist 1992; Kasumovic et al. 2008; Weir et al. 2011; Kappeler 2017; but see Klug et al. 2010; Monroe et al. 2016). Measuring variation in sexual selection requires considering the impact of local scale measures of sex ratio, group size, and composition. Most commonly, the strength and direction of sexual selection are assessed using (1) the sexual selection gradient or Bateman gradient, where the fitness gain (reproductive success, number of offspring) from having multiple partners (mating success, number of mates) is compared between males and females (Bateman 1948; Arnold and Wade 1984); (2) reproductive variance, how much the number of offspring varies among individuals of the same sex (Wade 1979); and (3) reproductive skew, the partitioning of reproduction among same-sex individuals within social groups (Sherman et al. 1995).

Changes in reproductive variance, reproductive skew, and the Bateman gradient at the local scale may in turn impact sex roles. Sex roles are usually defined by two traits: (1) differences in behavior, such as mate choice (courtship), mating competition (same sex aggressions), and mate guarding (e.g., Le Boeuf 1974; Arak 1983; Candolin 1999), and (2) sexual dimorphism, the difference between the sexes in a morphological trait (e.g., Berglund et al. 1986; Mori et al. 2017). These measures may change depending on extrinsic factors. For example, female water striders become less choosy when the sex ratio is higher, leading to less intense sexual selection on males (Arnqvist 1992). Similarly, female annual killifish change their mate choice throughout the mating season depending on the group sex ratio (Passos et al. 2014). In two-spotted gobies, agonistic behavior switches from male-male competition to female-female competition, and there was a reversal of courtship roles from male to female when male abundance declines and the OSR becomes more female biased (Forsgren et al. 2004). However, the extent to which sexual selection is variable and sex roles can be plastic in response to small-scale differences in the size and structure of local groups is rarely explored in wild populations and may shed light on factors constraining the evolution of male and female traits.

An interesting group in which to explore group-level differences in sexual selection is coral reef cardinalfishes (Apo gonidae). Adult cardinalfishes often live in groups, and many species show strong site fidelity and homing abilities (Marnane 2000; Døving et al. 2006; Gardiner and Jones 2016; Rueger et al. 2016a). Cardinalfishes are a family of paternal egg bearers that are often sex role reversed, with male mate

choice and female-female competition, but also show the potential for having sex roles that vary depending on social conditions (Kuwamura 1985; Okuda and Yanagisawa 1996; Okuda 1997, 1999a, 1999b). Pajama cardinalfish, *Sphaeramia nematoptera*, live in groups clearly defined by sheltering together in distinct branching coral heads. As nocturnal plankton feeders, group members leave at night to forage on sand flats but return to hide among the very same coral head branches every day (Rueger et al. 2014, 2016a). Pajama cardinalfish are associated with a partner of the opposite sex, and most pairs mate monogamously within groups (Rueger et al. 2019). Some extrapair mating by both males and females also occurs—when a male is mouthbrooding a whole clutch or part of a clutch whose genetic mother is a female other than the behavioral partner (cuckoldry) or when a female's eggs are detected in the mouth of a male that is not her behavioral partner (sneaker males; Rueger et al. 2019)—but at least 71% of both monogamous and extrapair mating events happen within the same group (Rueger et al. 2019). This indicates that the high degree of site attachment prevents most individuals from seeking a mating partner in a different group. These behaviors, together with the ease of sampling a whole clutch from a male's buccal cavity, facilitate measuring mating success (the number of reproductive partners) and reproductive success (the number of offspring produced) separately, and therefore the Bateman gradient as well as agonistic behaviors can be observed at the group level. The size and structure of social groups vary substantially in this species. Group size ranges from 2 to 25 individuals, with varying numbers of adults, subadults, and juveniles (Rueger et al. 2016b) and with varying ASRs. *Sphaeramia nematoptera*, therefore, is a good model system in which to investigate the consequences of variability in social group size and structure on sexual selection.

Here, we measure group size and group ASR within discrete social groups in a population of *S. nematoptera* from Papua New Guinea to investigate their effect on the strength and direction of sexual selection. Costly paternal mouthbrooding renders males the limiting sex and should make *S. nematoptera* a good candidate species to show reversed sex roles. Male cardinalfish cannot feed during mouthbrooding and require a lag period in between brooding a clutch, while females can produce eggs faster than males can brood them and have a higher potential reproductive rate (PRR; Kuwamura 1985; Okuda 1997). We would expect this to manifest as multiple mating by females, a steeper Bateman gradient, and stronger sexual selection on females, leading to greater female-female competition, with females being larger and more aggressive and potentially having some sexually dimorphic traits. Pajama cardinalfish have a dorsal fin filament that does not have an obvious ecological use and may function as an ornament, as other ornaments have been described in cardinalfishes (Okuda et al. 2003).

Variation in group size and ASRs within social groups may lead to different reproductive behaviors and strategies for both male and female individuals. Sexual selection theory predicts that the more access is limiting to either one of the sexes, the stronger the competition between individuals of the opposite sex, and the higher the variance in reproductive and mating success (Darwin 1871; Bateman 1948; Trivers 1972; Emlen and Oring 1977; Andersson 1994).

Therefore, we tested four related hypotheses regarding group composition and sexual selection. In a sample of 47 *S. nematoptera* from multiple groups that we collected and sexed, we measured body length, weight, and a potential ornament in the form of a dorsal fin filament. We predicted sexual dimorphism with females having a larger body size and a larger ornament than males (Kasumovic et al. 2008). In 168 adult *S. nematoptera* from 18 groups, we measured individual behaviors, reproductive output, and number of mates over five observational periods and predicted that if agonistic behaviors are a proxy of mating competition, they will be directed toward same-sex individuals and be higher for each sex when their sex ratio is biased (Forsgren et al. 2004). We also predicted a steeper Bateman gradient, higher reproductive variance, and greater reproductive skew for males when the sex ratio is male biased (limited access to females) and similarly for females when the sex ratio is female biased and an impact of group size on opportunities to monopolize matings (reproductive variance and skew). Large local group size might increase measures of sexual selection in which some individuals may better monopolize partners at higher encounter rates. Alternatively, sexual selection might decrease in large local groups, perhaps owing to a breakdown of dominance hierarchies when encounter rates with competitors are high.

Methods

Study System and Tagging

This study was conducted over five periods, spanning 2 years—October to November 2012 (observational period: 32 days), February to March 2013 (21 days), July to August 2013 (16 days), March to April 2014 (23 days), and September 2014 (9 days)—in Kimbe Bay, Papua New Guinea (5°30'S, 150°05'E). To reflect approximately the same observational time span and thus enable comparisons of reproductive output, data were grouped by year. Social groups of the pajama cardinalfish, *Sphaeramia nematoptera*, were defined as occupying the same distinct patch of the stony coral *Porites cylindrica* at a minimum distance of 2 m from another group (fig. 1a). A total of 379 individuals from 18 groups at 5–17 m depth on five reefs were included in the study (see map in Rueger et al. 2019). All fish were caught using hand nets and diluted clove oil solution (Munday and Wilson 1997) and individually marked using

visible implant elastomer tags (Northwest Marine Technology). For each individual, we recorded a unique identifier, life stage and sex (see “Group Size and Structure”), and behavior (see “Behavioral Observations”); collected fin clips (see “Measuring Mating and Reproductive Success”); and measured standard length (SL). Other morphological measures (weight and dorsal fin filament) were collected only from a smaller sample (see “Sexual Dimorphism”). This study was conducted in accordance with James Cook University Ethics Committee (approval A1847).

Group Size and Structure

The number of individual fish in 18 spatially different groups were counted, and adult and juvenile status were determined according to SL (Rueger et al. 2016b). Sex was determined by observing the distended buccal cavity during brooding (male) and bulging abdomen shortly before brooding (female) as well as pairing behavior (Rueger et al. 2016b, 2018). A total of 168 individuals in 18 groups were identified as adults according to SL, with the remaining 211 being juveniles or subadults. Detailed demographics, including ASR and adult group size (number of adults per group), were gathered for 18 groups (fig. 1b, 1c). For 10 groups, 3 years of observations were available. For four groups, 2 years of observations were available, and for another four groups, only 1 year of observations was available. Reproduction was observed for 121 adult individuals for 16 of the 18 groups: in one group, reproduction was observed in all 3 years; in nine groups, reproduction was observed in 2 years; and in six groups, reproduction was observed in only 1 year. All of the statistical analyses in this study were carried out in R (ver. 4.0.3; R Core Team 2020). To determine whether ASR and group size varied significantly between groups, we conducted a linear mixed model (LMM) analysis using the lme4 package (Bates et al. 2015). We used ASR and group size as response variables in two separate models, with group ID as fixed factor and year as random intercept. Significance tests for LMMs were performed by likelihood ratio tests of the full model with the effect in question tested against the model without the effect. No obvious deviations from homoscedasticity were detected by visually inspecting the residual plots. No outliers or high variance inflation factors were detected in any of the best-fit models, using the package performance (Lüdtke et al. 2021). Conditional and marginal R^2 were calculated using Nakagawa's R^2 in performance.

Sexual Dimorphism

A sample of 47 adult *S. nematoptera* were caught from multiple groups and euthanized to extract their gonads and

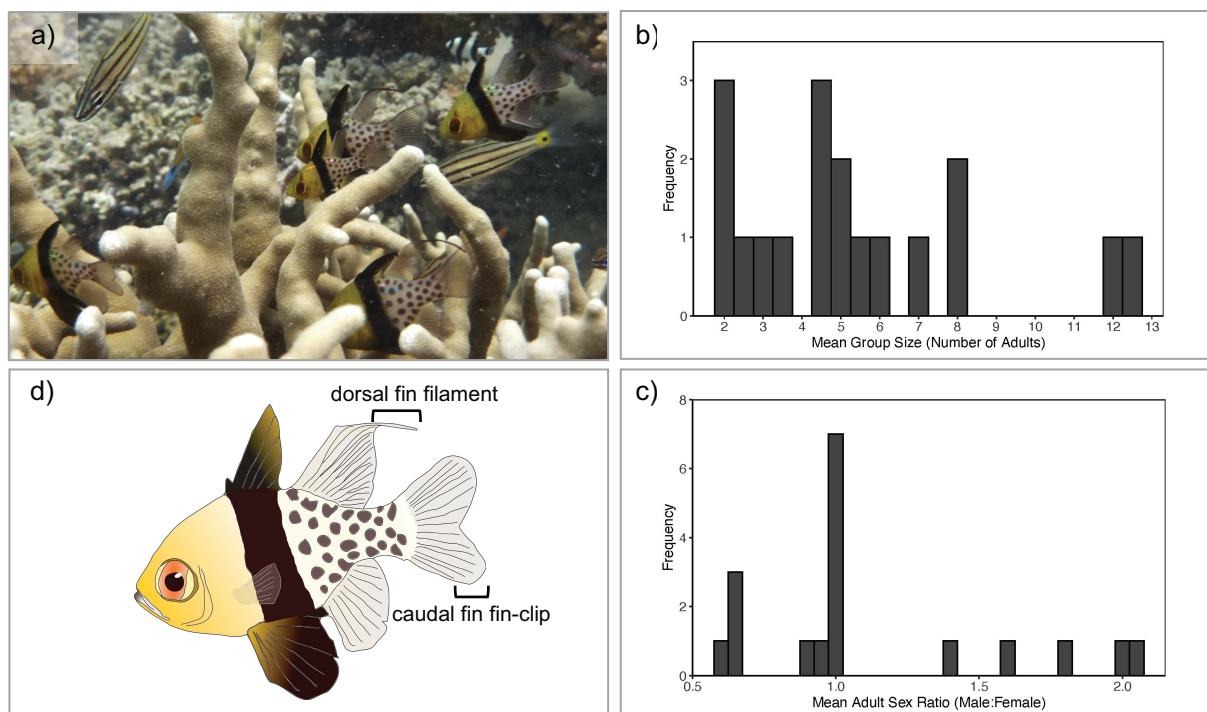


Figure 1: *a*, Photo of a group of *Sphaeramia nematoptera* in *Porites cylindrica* in Kimbe Bay, Papua New Guinea (credit: T. Rueger). *b*, *c*, Frequency of mean group size over all observational periods (*b*) and mean adult sex ratio over all observational periods (*c*) for groups of *S. nematoptera* ($N = 18$ groups). *d*, Schematic of *S. nematoptera*, including location of the dorsal fin filament and the caudal fin from where the fin clip was taken.

determine sex (see Rueger et al. 2018 for detailed methods). These samples were not part of the long-term monitoring effort described above for 18 groups but instead originated from separate groups. There were 20 females and 27 males in the sample. Their SL (fish length from the tip of the snout to the posterior end of the last vertebra, excluding the length of the caudal [tail] fin) and the length of the dorsal filament (fig. 1*d*) were measured to the nearest millimeter using calipers, and wet weight (mass) was measured using a digital scale to the nearest 0.1 g.

We conducted a directed comparison between three morphological traits—SL, mass, and dorsal fin filament—that are (1) highly distinguishable in this species (dorsal fin filament length; fig. 1*d*) and (2) commonly sexually dimorphic among other species (body size). While dorsal fin filament length may simply be represented by a univariate measurement (mm), body size, in contrast, is better represented as a multivariate morphological trait (Freeman and Jackson 1990). Therefore, we first constructed a multivariate metric of body size by loading SL (mm) and mass (g) into a principal component analysis (PCA) and extracting the first component from the PCA (the allometric size variable) for each individual (henceforth referred to as “body PC1”; explaining 97% of variance in SL and mass measurements). To aid in visual interpretation, PC1 values were normalized between

0 and 100. PCA was conducted in R using the function `prcomp`, and mass was not log transformed before loading into the PCA because both mass and dorsal filament scaled linearly (linear model: $\beta = 2.96$, $t = 10.10$, $df = 45$, $P < .001$, adjusted $R^2 = 0.687$). Sexual dimorphism in body size was tested using a linear model with body size (body PC1) as the Gaussian-distributed response variable and sex as the sole fixed effect predictor.

Sexual dimorphism in dorsal filament was tested using a similar linear model; however, body size was replaced with dorsal filament (mm) as the response variable (Gaussian distributed). To account for an influence of body size on dorsal filament alone and to test for differences in allometric scaling between body size and dorsal filament between sexes, we included sex (binomial), body size (numeric), and an interaction between sex and body size as fixed effect predictors.

The effects of either ASR or group size were not tested on measures of sexual dimorphism because these measures were not known for all of the groups the sampled fish originated from. A large part of the sample came from the same group (24 of 47), which had an ASR of 1.27 and was collected as a whole (all 24 adults). The size distribution for the 24 individuals in this group was similar to the other 23 fish included in this sample (see fig. S1; figs. S1–S9 are available online). The other 23 fish in the sample were

collected opportunistically from multiple groups of unknown ASR.

Behavioral Observations

During each of the five observational periods, all individuals from 18 groups were located every 2–3 days via visual census on SCUBA. On each day, we noted whether males were brooding and which individuals were in pairs (see Rueger et al. 2018 for details), and we recorded the subject and object of any agonistic behaviors (chases or bites) over 15–20 min. We compared the number of aggressions recorded per individual between males and females using a Fisher's exact test. To determine whether ASR or group size had an influence on the number of observed aggressions, we fitted a generalized LMM in lme4 (Bates et al. 2015) with the number of aggressions per group as response variable, number of adults and ASR as predictor variables, and group ID as random effect term. We used a Poisson error distribution and fitted an observation-level random effect term to account for overdispersion. To account for one potential environmental factor that could influence mating dynamics, we tested whether using coral size (the size of the coral head occupied by each distinct group) as a covariate improved the model. Including coral size did not improve the model, and we therefore did not include it in the final model (log-likelihood test of model with coral size vs. without coral size: $\chi^2 = 0.368$, $P = .544$).

Measuring Mating and Reproductive Success: Genetic Parentage Analysis

Relative mating and reproductive success were measured by analyzing the parentage of embryos and matching them to known adult individuals in the population. A DNA sample was taken from each individual ($N = 379$) in the 18 groups via a commonly used nonlethal protocol (Dietrich and Cunjak 2006; Beldade et al. 2016; O'Donnell et al. 2017), by clipping their caudal fin using surgical scissors (fig. 1d). The resulting fin clips were stored in high-grade 95% ethanol. The procedure was carried out during the same sampling effort (~90 min) for all of the group members at the beginning of the first study period. Caudal fin tissue grew back to the preprocedure size within 2–3 weeks, and no adverse effects on survival or behavior were recorded (T. Rueger, personal observation). Within the study periods, all broods were collected by catching brooding males. Clutches were then subsampled for genetic analysis, and individual embryos were stored in high-grade 95% ethanol. To consider the possibility of multiple mothers and fathers in each clutch, approximately 10 eggs were sampled from different parts of each egg mass, including several points on the

surface and the center of the congealed egg mass (see details in Rueger et al. 2019). A total of 1,056 embryos from 105 broods carried by 64 males were assayed.

All individuals were genotyped at 23 microsatellite loci with a range of 3–34 alleles observed per locus. Four markers that showed high genotyping error ($\geq 6\%$) were excluded from the analyses. The remaining 19 loci had an average genotyping error of $2.2\% \pm 0.4\%$ SE. Parentage assignments were conducted with COLONY (ver. 2.0; Jones and Wang 2010) to identify the most likely mother or mothers and father or fathers of the sampled eggs carried by male cardinalfish. We identified the number of genetic mates (mating success) and number of offspring (reproductive success) for each reproductive individual. Detailed methods of genotyping and parentage analysis as well as marker-specific statistics can be found in Rueger et al. (2015, 2019).

Bateman Gradient

We investigated the Bateman gradient by estimating the linear regression slope between mating success (number of genetic mates) and reproductive success (number of offspring assigned through parentage; Lande and Arnold 1983; Arnold and Wade 1984). We compared the slopes between males and females collating all data for each individual for the five observational periods and using Bayesian regression models (brms package; Bürkner 2017). To explore the influence of group size and structure, we calculated the mean number of adults for each group (fig. 1b) and the mean ASR for each group (fig. 1c) over all observational periods. We determined the relationship between mating success and reproductive success using group size (number of adults per group) and group ASR as continuous covariates and sex as a categorical covariate. We included the four-way interaction between number of mates, sex, ASR, and number of adults; the three-way interaction between number of mates, sex, and ASR; the three-way interaction between number of mates, sex, and number of adults; and the two-way interaction between number of mates and sex. To facilitate comparison with different species and group structures, we used standardized Bateman gradients, where we used the relative number of offspring and z -standardized number of mates (Schielzeth 2010; Collet et al. 2014). We also used z -standardized measures of ASR and adult group size. To account for non-independence of samples, we also included group ID as a grouping term. We used weakly informative priors. We ran the model for 10,000 iterations in each of four chains, with a 2,000-iteration warm-up. We confirmed model suitability using standard Bayesian diagnostic tests (leave-one-out [LOO] Pareto k : 97% < 0.5 , 3% 0.5–0.7; $\hat{R}$: all = 1; Neff ratio: all > 0.9 ; nondivergent trace plots; autocorrelation factor = low autocorrelation; density

plots = unimodal; see diagnostic plots in figs. S2, S3). LOO cross-validation results indicated that predictions in the fitted model were robust to pointwise out-of-sample replacement of data points. Because the response variable (number of offspring) consists of count data, we also used expected log-predicted density (ELPD) scores to compare the fit of the Gaussian linear regression model typically used in Bateman's gradient assessments with one with a Poisson distribution. The more typically used linear regression model fit the data better ($\Delta\text{ELPD} = -60.7$) and facilitates easier interpretation of the Bateman gradient; we therefore chose this model for further exploration. As above, we tested whether using coral size as a covariate improved the models. ELPD scores were lower when including coral size, and we therefore did not include it in the final models ($\Delta\text{ELPD} = -2.1$, $\text{SE} = 2.9$). Using the covariate model constructed from our observations, we compared Bateman gradient slopes (emmeans package; Lüdtke 2018) for males and females across nine hypothetical breeding groups with crossed levels of group size (low = 3, medium = 8, high = 15) and ASRs (male dominated = 1.9, balanced = 1, female dominated = 0.6). This counterfactual modeling is useful for identifying the causal implications of manipulating one or more predictor variables in lieu of experimental population manipulations, therefore showing the implied predictions for imaginary experiments (McElreath 2020). Model diagnostics are presented in figures S2 and S3.

Reproductive Variance

We quantified reproductive variance (sums of squares of differences from means in reproductive success as number of embryos assigned to each parent) across the full study period for each sex, overall, and within the 16 groups in which reproduction was observed. We used a Bayesian framework with a Gaussian distribution to model differences in within-group reproductive variance for each of the sexes in relation to the interacting effects of standardized group size and ASR. We standardized within-group measures of group size and ASR by subtracting the overall mean and dividing the result by the overall standard deviation of each variable, respectively (sensu Jones 2009). The interaction between standardized group size and ASR was removed from the reduced model after showing nonsignificance. Comparison of LOO ELPD scores showed this reduced model fit the data better than the full interaction model (difference > 2). As above, including coral size reduced model fit, and we therefore did not include it in the final models ($\Delta\text{ELPD} = -1.7$, $\text{SE} = 1.4$). Models were checked for fit as above. Estimated marginal means and credibility intervals (CIs) were extracted using emmeans (Lenth 2020) and plotted with ggeffects (Lüdtke 2018). Model diagnostic plots are shown in figure S4.

Reproductive Skew

To calculate skew in reproductive success, we used the multinomial index implemented in the SkewCalc package (Ross et al. 2020). The multinomial index (M) is related to Nonacs's binomial index B (Nonacs and Hager 2011) and accounts for heterogeneity in the number of observational periods in which an individual was observed (Ross et al. 2020). We calculated M for males, females, and the population as a whole, as well as for the different ASR and group size categories for each year. To explore the influence of group size and sex ratio on M for each of the 16 groups for each of the 3 years where reproduction was observed (number of groups reproducing: $N_{2012} = 6$, $N_{2013} = 11$, $N_{2014} = 13$), we assigned a categorical variable representing group size (small: $N_{2012} = 2$, $N_{2013} = 5$, $N_{2014} = 4$; medium: $N_{2012} = 2$, $N_{2013} = 3$, $N_{2014} = 8$; large: $N_{2012} = 2$, $N_{2013} = 3$, $N_{2014} = 1$) and a category for ASR (female biased < 1 : $N_{2012} = 1$, $N_{2013} = 4$, $N_{2014} = 3$; equal 1: $N_{2012} = 1$, $N_{2013} = 3$, $N_{2014} = 6$; male biased > 1 : $N_{2012} = 4$, $N_{2013} = 4$, $N_{2014} = 4$).

Results

Group Size and Structure

The ASR in the overall population was balanced with 88 females and 80 males (ratio = 0.91; exact binomial test: $P = .59$; fig. 1b). Between individual groups, the adult male-to-female sex ratio ranged from 0.6 to 2.1 (fig. 1b). The average adult group size of the 18 spatially different groups ranged from 2 to 12.7 individuals with a mean ($\pm\text{SE}$) of 5.39 ± 0.74 (fig. 1c). Both group size and ASR varied significantly across groups (LMM, log-likelihood test: for ASR, $\chi^2 = 26.45$, $\text{df} = 14$, $P = .023$; for group size, $\chi^2 = 41.68$, $\text{df} = 14$, $P < .001$; fig. S5).

Sexual Dimorphism

Body PC1 differed significantly between males and females (linear model: $\beta = -0.94$, $t = -2.49$, $\text{df} = 45$, $P = .017$, $R^2_{\text{adjusted}} = 0.102$; figs. S5, S6), with females being larger than males (figs. S6, S7). There was also a significant interaction between sex and body PC1 in explaining dorsal filament length (linear model: $\beta = -2.06$, $t = -2.15$, $\text{df} = 43$, $P = .037$, $R^2_{\text{adjusted}} = 0.074$; fig. S8), with females having longer dorsal filaments at larger body sizes.

Aggression

Of the 168 adults (88 females and 80 males) monitored from 15 groups, aggressive behaviors were observed for only 22 females (25%) and 14 males (17.5%). On average, females were twice as aggressive as males (Fisher's

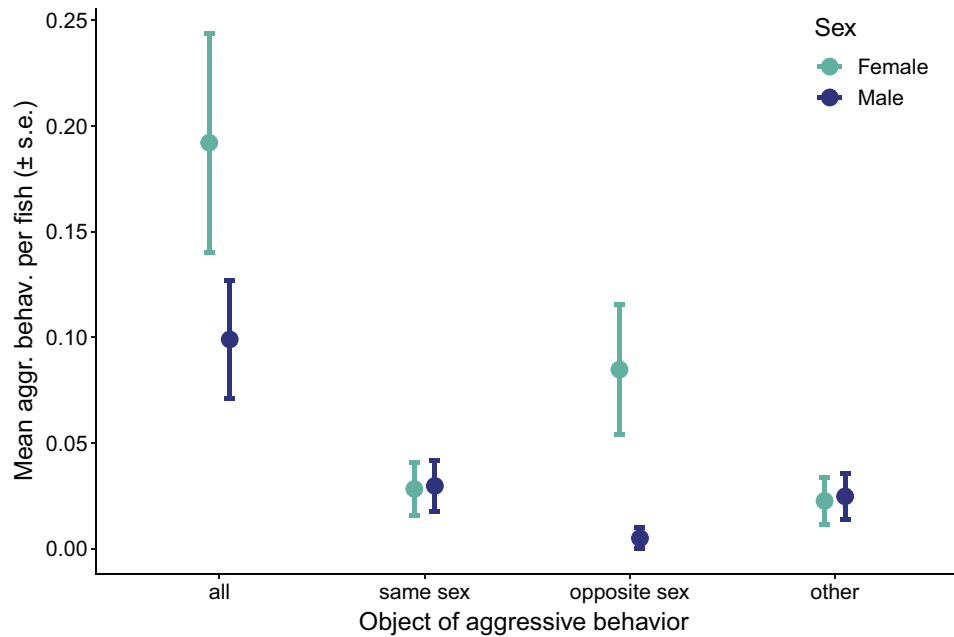


Figure 2: Mean number of aggressive behaviors ($\pm$ SE) observed per female ($N = 88$) and male ($N = 80$) pajama cardinalfish, *Sphaeramia nematoptera*, from 15 groups over the study period. Aggressive behaviors are displayed for the whole population combined (all) and categorized according to the receiver of the aggression (same-sex individual, opposite-sex individual, or juvenile/heterospecific individual [“other”]).

exact test: $P = .007$; fig. 2), and female aggression toward a conspecific of the opposite sex accounted for this difference. Half of all female aggression was toward conspecific males, whereas only one incident of aggression by a male toward a female conspecific was observed during the total 500 hours of observation (fig. 2). The other half of female aggression was divided between aggression toward conspecifics of the same sex and conspecific juveniles/heterospecifics at levels comparable to male aggression (fig. 2).

Both adult sex ratio and number of adults in the group had significant effects on the number of aggressive behaviors observed (marginal $R^2 = 0.268$), with more aggression performed when the sex ratio was more male biased (estimate $\pm$ SE = 1.589 ± 0.708 , $P = .025$) and when the group size was larger (estimate $\pm$ SE = 0.189 ± 0.083 , $P = .022$). The interaction between ASR and group size was not significant (estimate $\pm$ SE = -0.017 ± 0.149 , $P = .907$).

Bateman Gradient

In the entire sampled population across years, the standardized Bateman gradient was positive (estimated marginal slope = 0.82, 95% CI = 0.70 to 0.95; fig. 3) and did not differ between males and females (95% CI = -0.21 to 0.10; fig. 3). The model explained 73% of the variance in the data ($R^2 = 0.73$, 95% CI = 0.68 to 0.77).

Differences in standardized Bateman gradients for individuals exposed to different combinations of ASR and adult group size were statistically supported for both males (95% CI = -0.32 to -0.04) and females (95% CI = -0.44 to

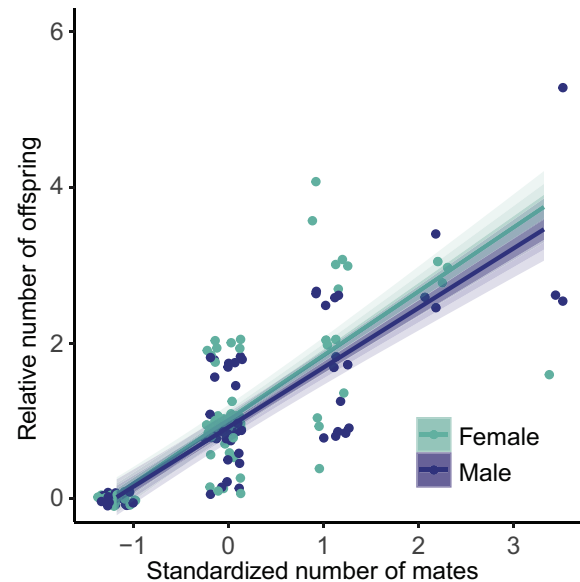


Figure 3: Standardized Bateman gradient (95% credibility interval) estimated for the pajama cardinalfish, *Sphaeramia nematoptera*, for all adult individuals ($N_{\text{groups}} = 18$, $N_{\text{indiv}} = 168$) by sex. Data points are jittered.

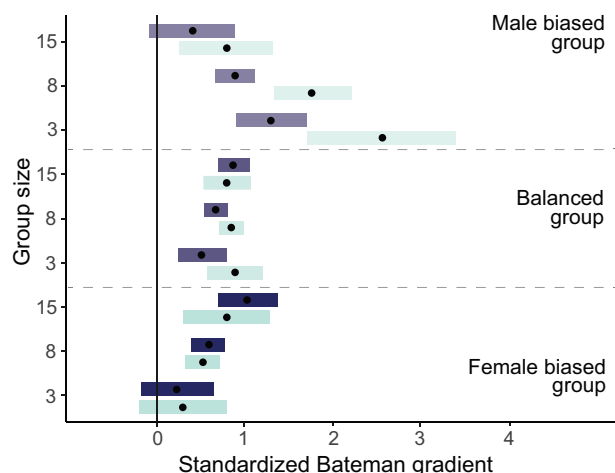


Figure 4: Standardized Bateman gradient as the slope between number of offspring produced (reproductive success) and number of mates acquired (mating success; 95% credibility intervals) estimated for pajama cardinalfish, *Sphaeramia nematoptera*. Males are shown in blue, and females are shown in green.

0.04). When sex ratios were male biased, female standardized Bateman gradients were steeper than those for males and male, and female Bateman gradients became steeper with decreasing group size (figs. 4, 5, S9). However, when groups were female biased, standardized Bateman gradients became steeper with increasing group size (figs. 4, 5, S5). When sex ratios were balanced, male standardized Bateman gradients became steeper with increasing group size but did not change for females (figs. 4, 5, S9). Overall, female standardized Bateman gradients were steeper than male standardized Bateman gradients only in small groups and balanced or male-biased groups (figs. 4–6).

Reproductive Variance

In the overall population, reproductive variance across all years was 131.42 for males and 90.64 for females, a ratio of 0.69. Across the 16 groups, reproductive variance was not lower in females than males overall (95% CI = −90.7 to 48.8) and was also not influenced by interactions between adult group size and sex ratio for either sex (95% CI for interaction term: male = −100 to 56.6, female = −65.5 to 87.0). When removing the sex ratio–group size interaction from the model, there was a better fit to the data (LOO Δ ELPD = −2.4) and evidence to suggest that adult group size and sex ratio may have a positive influence on reproductive variance in both sexes (with >90% and >75% credibility, respectively, for overall effects; fig. 7a). Sex differences in these effects were not significant (fig. 7). The model with sex interactions explained ~38% of reproductive variance ($R^2 = 0.38$, 95% CI = 0.13 to 0.61).

Reproductive Skew

Over the whole population, the posterior distribution of the multinomial index M , accounting for diminishing returns with age, showed a significant reproductive skew for males (mean $M = 0.12$, 95% CI = 0.05 to 0.21), females (mean $M = 0.14$, 95% CI = 0.06 to 0.23), and for the overall population for the sexes combined (mean $M = 0.14$, 95% CI = 0.07 to 0.19).

Reproductive skew was significant in groups with female- and male-biased ASRs (female-biased ASR: mean $M = 0.14$, 95% CI = 0.05 to 0.26; male-biased ASR: mean $M = 0.09$, 95% CI = 0.02 to 0.18) but was not significant in groups with an equal ASR (mean $M = 0.10$, 95% CI = −0.01 to 0.24). There were no differences between sexes dependent on ASR.

Reproductive skew was significant at all group sizes, with the highest skew at medium group sizes (small: mean $M = 0.11$, 95% CI = 0.01 to 0.28; medium: mean $M = 0.17$, 95% CI = 0.07 to 0.29; large: mean $M = 0.11$, 95% CI = 0.03 to 0.21). There were no differences in reproductive skew between the sexes as a function of group size.

Discussion

Our findings confirm that for the mouthbrooding pajama cardinalfish, the strength of sexual selection depends on the size and structure of the local social group, a result that would not have been evident by focusing only on the larger population level. In agreement with our predictions, females were larger than males and had a larger dorsal fin filament, likely an ornament favored by sexual selection acting on females at the population level. The Bateman gradient and reproductive variances of males increased as the ASR became more male biased; however, contrary to our predictions, these measures also increased for females as the ASR became more male, not female, biased. In terms of group size, we proposed two predictions, and we found evidence for both depending on the ASR of the group. Bateman gradients of both sexes became steeper with decreasing, not increasing, local group size but only in male-biased groups. When local groups were female biased, only male, not female, Bateman gradients became steeper with increasing group size, and in general, reproductive variances of males, but not females, increased with increasing group size across all ASRs. In summary, whereas ASR impacts the strength of selection of both sexes regardless of group size, group size drives sexual selection differently depending on group ASR and differently between the sexes. Our results demonstrate that assessing the strength and direction of sexual selection requires considering small-scale variability in both group size and sex ratio.

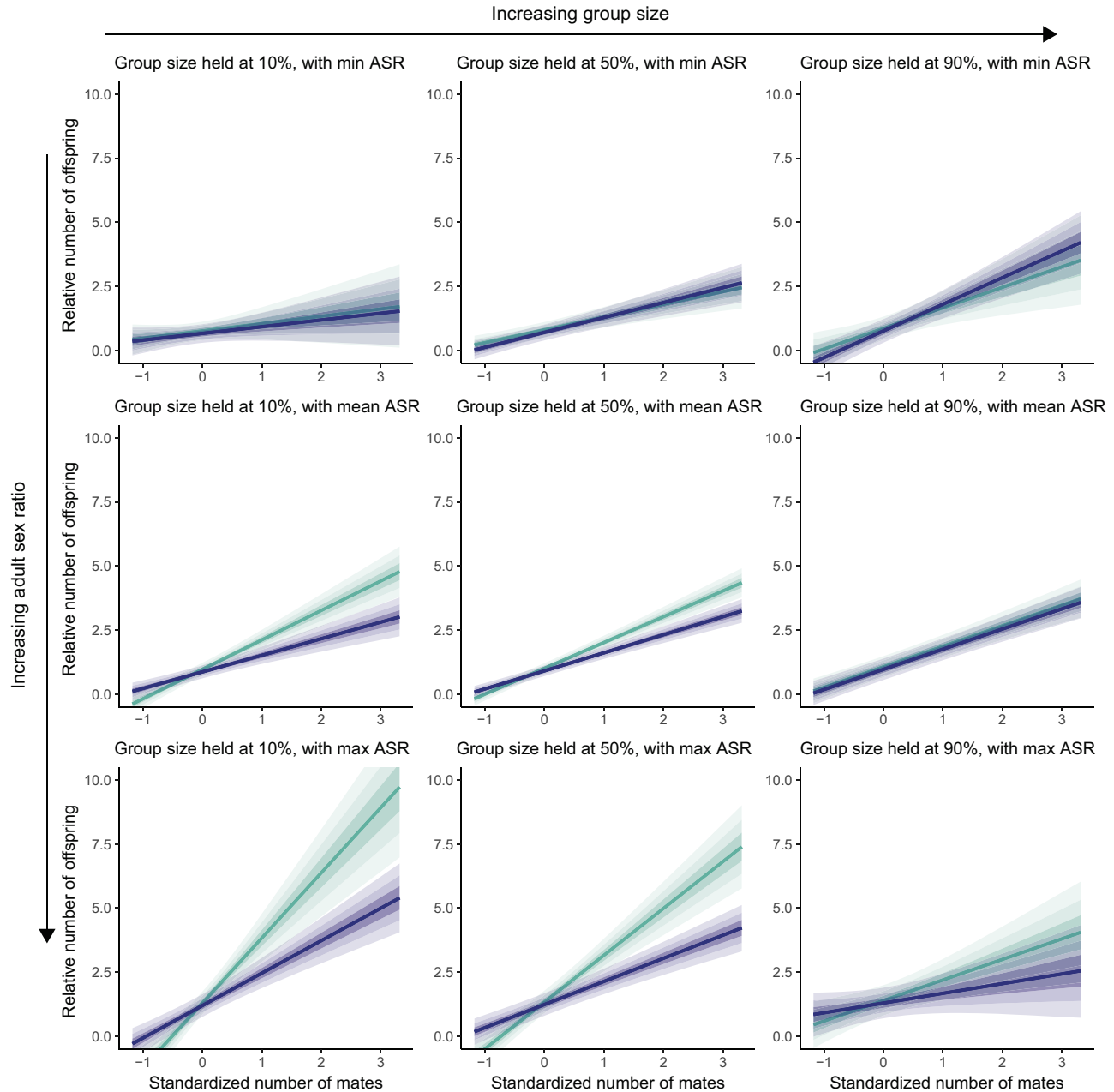


Figure 5: Log-linear estimates of the relationship between number of offspring (log) and number of mates at interacting levels of adult group size and adult sex ratios (ASRs) for male (blue) and female (green) *Sphaeramia nematoptera*.

Because of the presence of costly paternal mouth-brooding, we predicted multiple mating by females, a steeper Bateman gradient, and stronger sexual selection resulting in females being the larger sex, being more aggressive, and having a sexual ornament. Our findings agree only partially with these hypotheses because of the variability in the strength of sexual selection with group size and ASR. Female Bateman gradients were steeper than males, indicating

stronger sexual selection on females, but only in small and medium-sized groups with male-biased or equal sex ratios. When sex ratios were male biased in small and medium groups, and to a lesser extent in large groups, gradients of both sexes were steeper and reproductive variances were higher. From the males' perspective, this is likely due to higher proportions of males not having the opportunity to brood eggs when there is a limited number of females.

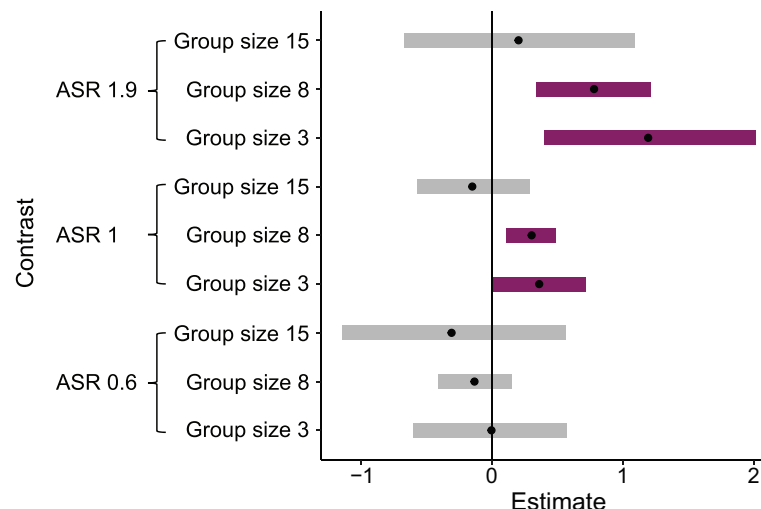


Figure 6: Custom contrasts between male and female Bateman gradients at three adult sex ratios (ASR; female biased = 0.6, equal = 1, male biased = 1.9) and three group sizes (3, 8, 15 adults).

From the females' perspective, male-biased sex ratios provide more opportunities for multiple matings outside of monogamous pairs. Promiscuity drives strong sexual selection in other socially monogamous taxa (Webster et al. 2007). Meanwhile, the increasing potential for sexual selection on both sexes when ASR is male biased results in size-assortative mating (Rueger et al. 2018).

On the basis of high parental investment, we predicted there to be sexual dimorphism in *S. nematoptera*, and we

did find that females were larger than males and showed signs of longer dorsal fin filaments. This could be the result of stronger sexual selection in male-biased groups, as indicated by the steep Bateman gradients for females in these groups, although the current study cannot establish causation and cannot exclude natural selection as a driver. Aggression in *Sphaeramia nematoptera* was also higher in females than males, especially in male-biased groups, and females were larger. However, in contrast to our

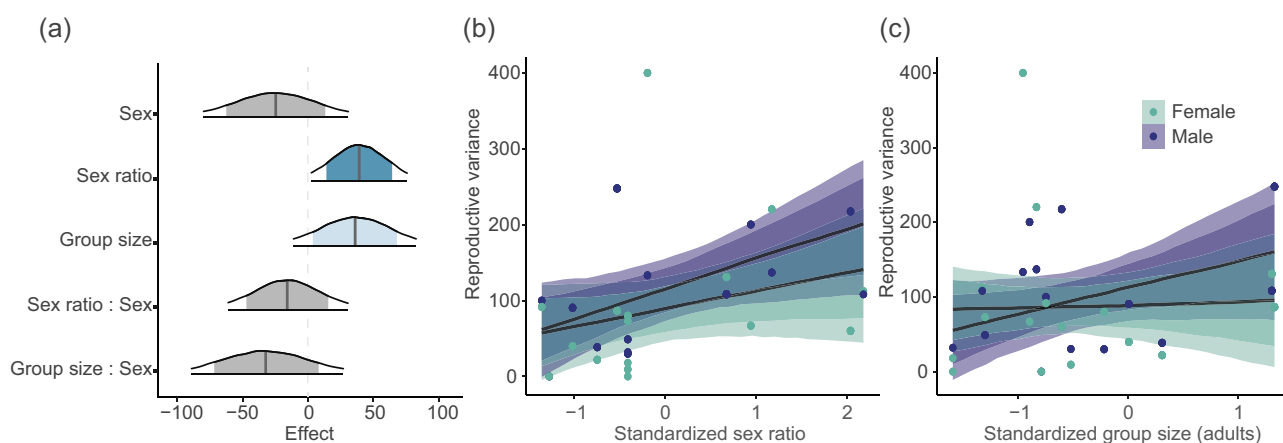


Figure 7: *a*, Predicted effect estimates for reproductive variance (60% and 90% credibility intervals) in pajama cardinalfish, *Sphaeramia nematoptera*. Dark blue indicates effects supported with >90% credibility. Light blue indicates trends supported with >75% credibility. Gray indicates effects not supported with 75% credibility. *b*, Predicted reproductive variance (60% and 90% credibility intervals) for standardized sex ratios (from female biased to male biased) in female and male *S. nematoptera*. *c*, Predicted reproductive variance (60% and 90% credibility intervals) for standardized group sizes (from small to large) in female and male *S. nematoptera*.

expectation of female-female competition, female aggression in *S. nematoptera* was largely directed toward males, not females. Thus, female aggressive behavior appears not to serve in mating competition or in guarding high-quality mates but could represent attempts to defend against male sneak mating and egg predation, both of which have been reported in this species (Rueger et al. 2019). On the other hand, aggressive behavior and sexual size dimorphism may not be related with sexual selection but instead may result from natural selection. Male cardinalfish are limited in their foraging during brooding, which may constrain their growth rate compared with females, leading to size dimorphism. Female aggressiveness may be selected in a context of competition for food or for refuges in the coral reef. While some cardinalfishes have been labeled sex role reversed (Kuwamura 1985; Okuda 1997), detailed data on reproductive variance and skew are often lacking, and these may be needed to fully understand sexual selection in this family. Our data suggest that measuring only parental care strategies (mouthbrooding) does not predict the strength and direction of sexual selection, and small-scale demographic factors at the level of the social group, such as ASR and group size, also need to be considered (Alonzo 2010; Kasumovic et al. 2008; Safari and Goymann 2020).

Group living, especially in large groups, may provide cardinalfish with more opportunities for multiple matings because of a higher rate of mate encounter, as is the case in other group-living animals (Kokko and Johnstone 2002; Kokko and Rankin 2006). This results in sexual selection varying in relation to group size (i.e., how many partners are locally available). In cardinalfishes, females have higher PRR because they can produce eggs more quickly than males can brood them, thus benefiting from polygamous matings (Kuwamura 1985; Okuda 1997). However, males can cannibalize partial or whole broods (Okuda and Yanagisawa 1996) and perform sneak matings, thus also benefiting from polygamous matings (Rueger et al. 2019). In the present study the Bateman gradients of both males and females became steeper as group size increased, especially for males, but only when the sex ratio was female biased. In this study it seems that some males benefited from the higher encounter rates with not only females but also males in large groups, perhaps by performing more sneak matings. Interestingly, female reproductive variance also increased with increasing group size. As female cardinalfishes often have higher PRR, rendering males the limiting sex, large-group living provided female cardinalfish with more opportunities for multiple polygamous matings (Kuwamura 1985; Okuda 1997). However, when the groups were male biased, sexual selection (or Bateman gradients) of both sexes decreased at large group sizes. The reproductive variance of females in male-biased groups did not increase with group size, suggesting that females were producing

the same number of offspring but spread across a larger number of male partners, potentially due to their monogamous social partner being less able to prevent sneak matings from other males at high densities, decreasing the female Bateman gradient. On the other hand, in male-biased groups, although male reproductive variance increased with group size, offspring fathered by successful males were produced by a larger number of female partners, decreasing the Bateman gradient and reducing the fitness gain from having multiple partners.

Migration between groups may dampen the effect of small-scale demographics, and short-term migration between groups and matings among distant groups were previously demonstrated in *S. nematoptera* (Rueger et al. 2019). Nevertheless, most mating events (71%) take place within social groups (Rueger et al. 2019), and individuals often stay at the same site (coral head patch) for years (Rueger et al. 2018), emphasizing the importance of local group structure. Previous work has suggested that sex roles may be flexible and can change depending on demographic factors such as the OSR or ASR (Jones et al. 2000; Mills et al. 2007; Kokko and Jennions 2008b). Our results highlight that group size is another potentially important factor in determining sexual selection and the evolution of sex roles. Indeed, other cardinalfish species that were identified to have equal or variable sex roles, *Apogon notatus* and *Pterapogon kauderni*, are also gregarious species (Okuda et al. 2003; Vagelli and Volpedo 2004), whereas the ones identified as sex role reversed, *Apogon doederleini* and *Apogon niger*, are solitary or pair living (Kuwamura 1985; Okuda 1997). Future empirical studies across cardinalfish species should focus on manipulating group size alongside more well-known factors such as ASR to test the extent of this plasticity within species.

Our results highlight the importance of the sex ratio and size of groups on sexual selection; however, differences in sexual selection could also be due to environment factors. For example, differing light levels at different depths or areas of turbidity or dense vegetation may impact visual acuity and therefore mate choice directly (Endler and Houde 1995; Candolin et al. 2007, 2016). Depth may also impact group composition, as shown in the reef herbivore *Sparisoma viride*, where differences were found below 3 m compared with above 3 m, likely because of the impact of strong winds in shallow waters (van Rooij et al. 1996). As our focal groups were found between 5 and 17 m, surface winds will not impact group composition, and relatively small variation in depth, which does not consistently impact light levels or turbidity in coral reefs, is unlikely to play a substantial role in group composition, as found in other species (Krause et al. 2000; Balshine et al. 2001). Furthermore, *S. nematoptera* are nocturnal plankton feeders that make a nightly depth migration to at least 35 m, and as

such, the variation in depth between our focal groups during the day is unlikely to affect group composition. On the other hand, another environmental factor, size of available habitat patch, may impact both local population density and mating behavior (Arnqvist 1992; Kwiatkowski and Sullivan 2002). In coral reef fish the relationship between habitat patch size, social group, and mating system is variable between species, being more important in species with more specialized patterns of habitat use (Thompson et al. 2007). We tested the size of the coral that groups occupied as a potential covariate in our models but found that it decreased model fit for aggression, Bateman gradient, and reproductive variance, showing that patch size does not determine sexual selection in this cardinalfish species. We did not measure other environmental factors such as turbidity, predator density, and local temperature. They will be important to consider in future studies to tease apart the impacts of group composition and the environment on sexual selection.

Future Directions

Several interesting questions arise from our results. First, how will sexual selection operate at the population level in pajama cardinalfish if there are fluctuations in the strength and direction of selection, simultaneous sexual selection on both males and female (equal and considerable reproductive variances and skews), and significant Bateman gradients in both sexes? The influence of selection on heritable traits, such as body size and dorsal filament length, will be weaker at the level of the population in species in which the strength and direction of sexual selection vary spatially and temporally within a population. Testing how body size and dorsal filaments impact fitness and observing mating behavior directly to determine choosiness and mating competition will give further insight into the sex roles in this species. Second, does developmental phenotypic plasticity in traits evolve as a function of the demographics of the group into which a juvenile has settled (which ultimately determines the direction and strength of sexual selection) and the sex of each juvenile? Alternatively, male and female juveniles might select different groups in which to settle as a function of group demographics such that they develop within a group to which they are optimally adapted. Third, given that this species can move between groups (Rueger et al. 2016a), can their movement be explained by the sex ratios or group sizes of the original and new groups coupled with individual attributes? Also, does eviction or acceptance of newly settled juveniles or group-switching adults occur to prevent any change in or reinforce group size or sex ratio to which they may already be adapted to? Furthermore, what are the causal factors determining variation in demographic

factors at the social unit of the group? And last, does local environmental quality, such as live coral cover, correlate with group size, and how will anthropogenic climate change impact group size in this species and ultimately sexual selection?

Conclusion

Our study shows that local factors such as group size and ASR are crucial in determining the strengths and outcomes of sexual selection. We found that local group composition, rather than population composition, influences the strength of sexual selection in *S. nematoptera*. In this socially monogamous species, opportunities for extrapair matings, dictated by group size and sex ratio, drive strong sexual selection in both sexes. We find that Bateman gradient, reproductive variances, and levels of aggression all differed depending on group size and/or ASR. Only by measuring all of these factors and comparing them with demographic changes were we able to gain a complete picture of the complexity of sexual selection in this species. Clearly, parental investment, mating system, and demographic factors interact to inform sexual selection in cardinalfishes. It is likely that these findings hold true for many other species with high site fidelity in small social groups.

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Statement of Authorship

T.R. and S.C.M. conceptualized the study and acquired funding. T.R., N.M.G., and G.P.J. developed methods and designed the experiment. T.R. collected data. T.R. and H.B.H. performed molecular lab work and data analysis. T.R. and C.M. conducted statistical modeling, validation, and visualization. T.R. and S.C.M. wrote the original draft of the manuscript, and all authors contributed to reviewing and editing.

Data and Code Availability

Data and code are accessible at the Dryad Digital Repository (<https://doi.org/10.5061/dryad.tdz08kq4h>; Rueger et al. 2024).

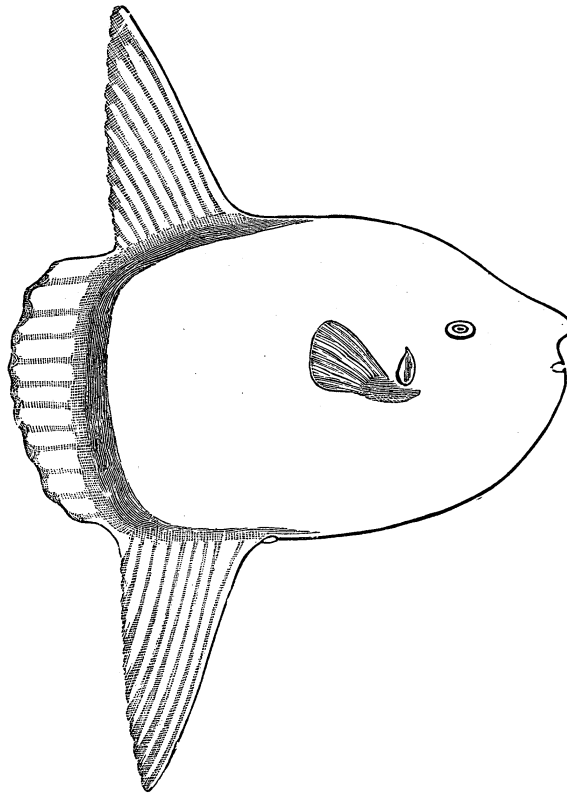
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"*Orthogoriscus mola* (adult, greatly reduced)." From the report on F. W. Putnam's communication "On the young of *Orthogoriscus mola*" at the Nineteenth Meeting of the American Association for the Advancement of Science, held at Troy, New York, August 17–24, 1870 (*The American Naturalist*, 1870, 4:629–640).