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Parentage analysis across age cohorts reveals sex differences in reproductive skew in a group-living cichlid fish, *Neolamprologus multifasciatus*

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Abstract

Group-living animals are often faced with complex reproductive decisions, namely how to partition within-group reproduction, how to obtain extra-group reproduction and how these two means of reproduction should be balanced. The solutions to these questions can be difficult to predict because ecological conditions can affect the scopes for within-group and extra-group reproduction in complex ways. For example, individuals that are restricted from moving freely around their habitats may have limited extra-group reproductive opportunities, but at the same time, groups may live in close proximity to one another, which could potentially have the opposite effect. The group-living cichlid fish *Neolamprologus multifasciatus* experiences such ecological conditions, and we conducted an intensive genetic parentage analysis to investigate how reproduction is distributed within and among groups for both males and females. We found that cohabiting males live in "high-skew" societies, where dominant males monopolize the majority of within-group reproduction, while females live in "low-skew" societies, where multiple females can produce offspring concurrently. Despite extremely short distances separating groups, we inferred only very low levels of extra-group reproduction, suggesting that subordinate males have very limited reproductive opportunities. A strength of our parentage analysis lies in its inclusion of individuals that spanned a wide age range, from young fry to adults. We outline the logistical circumstances when very young offspring may not always be accessible to parentage researchers, and present strategies to overcome the challenges of inferring mating patterns from a wide age range of offspring.

KEYWORDS
cichlid, ecological constraints, extra-group reproduction, group-living, microsatellite genotyping, reproductive skew

1 | INTRODUCTION

One of the greatest sources of conflict that group-living animals face is how they should share reproduction among group members. Across species, breeding groups can vary widely in how much reproductive sharing they engage in (Buston et al., 2007; Emlen, 1982a, 1982b; Johnstone, 2000; Reeve & Shen, 2013; Sherman et al., 1995). At one extreme, in so-called "high-skew" societies, reproduction within groups is monopolized by one or a few dominant individuals (e.g., naked mole-rat, *Heterocephalus glaber*, Clarke & Faulkes, 1998), while at the other extreme, in so-called "low-skew" societies, reproduction is shared rather equally across adult group members (e.g., banded mongoose, *Mungos mungo*, De Luca & Ginsberg, 2001; groove-billed ani, *Crotophaga sulcirostris*, Vehrencamp, 1983). How much reproductive inequality there exists among same-sex group members should depend on the benefits of group membership versus opportunities for independent breeding, the ability of individuals to compete for the chance to breed, and the ability of individuals to evict others from the group (Cant & Johnstone, 2009; Vehrencamp, 1983).

A species' ecology can profoundly affect the patterns of reproduction that occur within and between groups, though different outcomes may be expected depending on what ecological conditions are prevalent. For example, in saturated habitats where reproductive opportunities, such as alternative breeding sites, are rare outside the current group, subordinates pose little abandonment threat and so dominants that benefit from having subordinates in a group may not be forced to grant them within-group reproduction to stay (Cant, 2011; Cant & Johnstone, 2009). Furthermore, when free movement is constrained, for example due to high predation risk, it can become costly to prospect for reproductive opportunities elsewhere and so the payoffs of seeking extra-group reproduction are reduced (Petrie & Kempenaers, 1998). High population densities can also generate complex patterns of reproductive partitioning (Kokko & Rankin, 2006). Close proximity between groups living under high densities could facilitate extra-group reproduction between neighbours (Mayer & Pasinelli, 2013; Mougeot, 2004; Westneat & Sherman, 1997), yet this possibility has been poorly integrated into studies of reproductive skew to date (Riehl, 2017). High population densities, such as saturated habitats, also imply a shortage of vacant breeding territories, again limiting outside options for independent reproduction. Therefore, to better understand the costs and benefits of group-living, it is important to characterize how reproduction is partitioned among individuals within and between groups under a range of ecological scenarios.

Reproductive sharing is typically studied by inferring parentage from very young offspring. Sampling young offspring is desirable because they typically have not yet dispersed away from their parents or natal groups, making it easier to completely sample broods and to detect their parents amongst the nearby adults. However, in certain species complete sampling of very young offspring is not possible if, for example, the offspring are cryptic or elusive (Anderson et al., 2011) or if sampling would be invasive to the nest or breeding site. Yet, asking questions about reproductive division is still important for understanding the selection pressures that these animals face. In such cases, it might be necessary to infer parentage from a wider range of individuals in the population, including dependent offspring, independent juveniles and young adults. However, this poses several notable challenges. First, as time passes since the production of offspring, the odds increase that parents die due to natural mortality, making it less likely that the parents of older offspring will be found within a population sample. Second, as time passes, the odds also increase that parents and offspring have moved apart from one another. Thus, not only does a large enough area have to be sampled to ensure that both parents and offspring can be captured in the same population sample, but it also presents the problem of distinguishing between offspring movement, adult movement and extra-group reproduction. Third, as more time passes, there is increasing scope for temporal fluctuations in environmental conditions to impact mating decisions and patterns of parentage. Overcoming these hurdles poses an interesting challenge for empiricists seeking to investigate parentage in less traditionally suited study systems where dependent offspring are difficult to access.

Historically, reproductive sharing has been studied from a male perspective. This is probably due to the fact that males often express conspicuous competitive traits, and female fecundity imposes limits on the number of eggs that can be fertilized, or shared, in any given mating event. Yet, reproductive inequality among females can also be intense, as seen in cooperatively breeding species of African starling (Rubenstein & Lovette, 2009). Among primates, dominant females may reproductively suppress subordinate females or commit infanticide, thereby leading to pronounced reproductive skew among females (e.g., Beehner & Lu, 2013; Saltzman et al., 2009). The degree to which females experience reproductive skew has been largely studied in avian and mammalian taxa (Raihani & Clutton-Brock, 2010), leaving other taxa relatively neglected. Thus, there is a need to more broadly quantify the degree of reproductive sharing among females alongside that in males, and doing so will help clarify whether the same underlying principles operating on males are also at play for females.

Here, we conducted an intensive parentage analysis to investigate the distributions of male and female reproduction within and among wild groups of *Neolamprologus multifasciatus*, a highly social African cichlid fish from Lake Tanganyika. Groups of *N. multifasciatus*, which can consist of multiple males and multiple females, control small territories on the lake floor that contain collections of empty snail shells that have been excavated from the sand. The fish use these shells as shelters and breeding chambers where females lay eggs and care for offspring. Very young offspring do not leave their natal shells and are therefore extremely difficult to capture without environmentally destructive sampling by breaking open their shells. Thus, our sampling and parentage analyses primarily involved older juveniles, but also included young offspring and young adults where possible.

The ecological conditions that *N. multifasciatus*, and other shell-dwelling cichlids, experience are unique in that they could foreseeably hinder *and* facilitate extra-group reproduction. These small-bodied fish are particularly vulnerable to predators that roam the pelagic zone immediately above their territories, especially when moving beyond the safety of their shell shelters (Schradin &

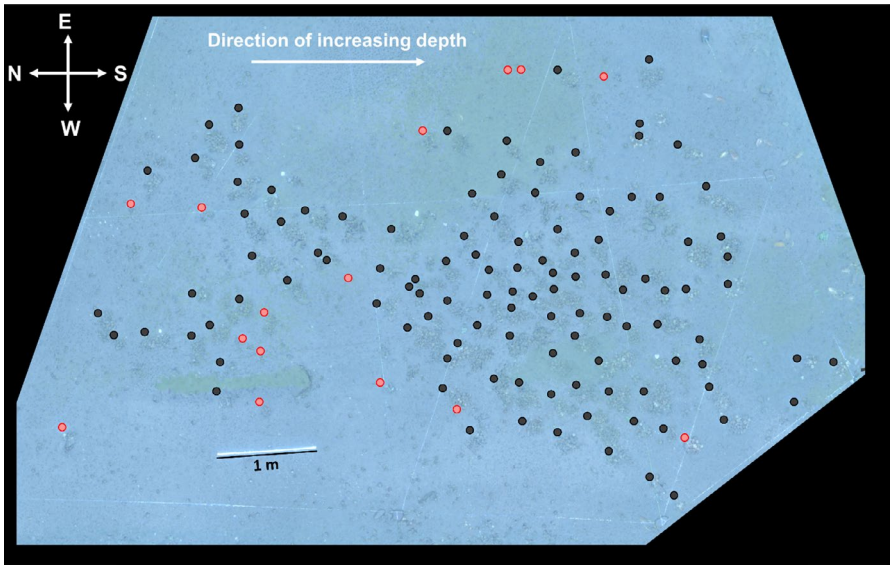

FIGURE 1 Map of study quadrat at depth of 10–11 m. Dots indicate the positions of *Neolamprologus multifasciatus* territories. Black dots indicate multi-individual groups. Red dots indicate solitary males. Note that this colony of *N. multifasciatus* territories was surrounded by a stretch of bare sand, at least 1 m in width, separating it from the rest of the shell bed. Approximate cardinal directions are indicated on the map

Lamprecht, 2000; A.B., L.K. and A.J., personal observations). This vulnerability potentially restricts their ability to prospect at neighbouring groups for reproductive opportunities. At the same time *N. multifasciatus* groups can be extremely abundant, often with no more than 30 cm separating groups (Jordan et al., 2016), and this density might support extra-group mating opportunities. To study reproductive sharing within each sex and the factors associated with skew, we took a holistic approach where in addition to assessing how much reproduction is secured by same-group or other-group individuals, we also examined how reproductive success and outside options for independent breeding change as adults age and grow larger. We began by asking whether the opportunity for independent breeding is constrained, and we did this by examining the frequency of newly established territories, the sizes of the solitary individuals that held them and where these territories were located within the *N. multifasciatus* colony. We then used genetic parentage analyses to examine the distributions of reproductive success among adult males and females and to assess when offspring disperse from their natal groups. We evaluate our results with respect to the monopolization of reproduction by dominant individuals, reproductive sharing within groups and opportunities for extra-group reproduction. We also discuss and offer solutions regarding the inference of mating patterns from parentage that may also be affected by changes in group composition since the time of spawning.

2 | METHODS

2.1 | Study species

Neolamprologus multifasciatus is one of the smallest Lamprologine cichlids endemic to Lake Tanganyika, East Africa (Konings, 2019; Lein & Jordan, 2021). *N. multifasciatus* live in social groups on the lake floor, on so-called "shell beds," regions where large accumulations of empty gastropod shells cover the sediment at extreme densities. Groups consist of a dominant male along with a number of reproductively mature females and subordinate males, as well as immature juvenile individuals (Kohler, 1998). The largest male in each group is always the dominant male as they act highly aggressively towards outsiders, engage in peacekeeping behaviours with their group members and patrol the widest areas of their territory (Bose et al., 2021; Gübel et al., 2021; Kohler, 1998). Body size variation is narrower among cohabiting females, but larger more aggressive females are considered dominant over other females (Schradin & Lamprecht, 2002). Each fish possesses a "home shell" within the group's territory—a shell that they return to regularly for shelter and where females lay their eggs and care for offspring (Gübel et al., 2021). Females lay small brood sizes, typically around six eggs (A.B. and A.J., personal observations; Kohler, 1998), and if fry are removed after hatching, females held under laboratory conditions can produce broods at intervals of ~3 weeks (Kohler, 1998). While dominant males traverse the entire territory, spanning areas up to ~100 cm^2, other adult group members partition the territory into individual, nonoverlapping, "subterritories" (Bose et al., 2021). Territory defence is largely conducted by the dominant male, but females and subordinate males can also assist (Kohler, 1998) particularly when intruders approach their subterritories. Thus, dominant males may acquire group augmentation benefits by tolerating subordinate males in their groups, though such benefits have yet to be explicitly studied. New territories are established by single males venturing away from their resident territory and excavating shells nearby, retreating back to shelter when approached by predators (Jordan et al., 2016). Once several shells have been excavated, the solitary male can attract females and grow his breeding group. However, little is known about how constraints might limit where new territories are founded or which males seek to create new territories.

2.2 | Field sampling

Between September and October 2019, while scuba diving, we identified all *N. multifasciatus* territories within a quadrat

(~10 × 10 m, depth range: 10–11 m) located in a wide-spanning shell-bed on the floor of Lake Tanganyika (near Mutondwe Island, 8°42′49.0″S, 31°07′22.9″E). This quadrat enclosed a cluster of territories (henceforth called "colony") separated from the rest of the shell bed by a border of open sand that was at least 1 m in width. The group sizes, territory densities, resource availability, substrate type and species community within this quadrat were all typical of the conditions experienced by *N. multifasciatus* in other regions in the wild (A.B., L.K. and A.J., personal observations). We used video from a swim-over of the quadrat (GoPro Hero 7 camera set to 1080-pixel resolution, 30 fps and a "linear" field of view) to recreate the layout of the study area (Figure 1) using Structure-from-Motion photogrammetry (Westoby et al., 2012). We then measured all pairwise distances between the *N. multifasciatus* territories in the quadrat based on their Cartesian coordinates as placed in IMAGEJ (version 1.53e). We also calculated the centroid of the colony by averaging the *X* and *Y* coordinates of all territories. During the field season, we systematically sampled all individuals from each territory in the quadrat. Because individual fish hide in their home shells when approached by a predator or diver, they can be captured simply by picking up the shells in a territory. We extracted the fish from their shells and sedated them with clove oil. The fish were sexed by inspection of their urogenital papillae, measured for standard length (cm, SL), and recorded as either adults or juveniles based on the presence of distinct banding along the sides of the body denoting sexual maturity (Kohler, 1998). The largest males in each group were recorded as the dominant males, while other males were recorded as subordinates (Kohler, 1998). We recorded which females were the largest in their groups, and labelled them as dominant females. Note that some groups had no clear dominant females (if there was only one female in the group or if all females were equal in size) and other groups could have multiple dominant females (if there were several size-matched, largest females). When possible, we sampled dependent offspring (henceforth, "fry"). Fry are very small, less than ~0.9 cm SL, and spend most of their time hiding in shells (Kohler, 1998), making them difficult to capture without breaking open the shells, which we did not do in this study. We therefore only sampled fry opportunistically. Large fish (>1.7 cm SL) were fin-clipped on their anal fins (taking at most 2 × 2 mm of tissue) and, when fully recovered from sedation, they were returned along with their shells to their original territories. Fish smaller than 1.7 cm SL (~20% of our whole sample) were euthanized with an overdose of clove oil (Neiffer & Stamper, 2009) and sampled whole due to the relatively large amount of tissue that clipping would have removed. All fin clips and whole fish were stored in 99% ethanol for later genetic analysis. In the days following each group's sampling, we would return and check for unclipped fish that had been missed on the original pass. Each group was checked on at least two further occasions, with unclipped fish becoming exceedingly rare on subsequent checks, thereby lending high confidence that we had sampled all, or nearly all, individuals living in the quadrat. In total, we sampled 835 fish (239 adult females, 191 adult males, 382 juveniles and 23 fry) from 128 territories, constituting all the territories in the quadrat. This work was carried out with permission from the Fisheries Department of Zambia under study permits issued by the government of Zambia (No. G7067690 and C3195368).

2.3 | Microsatellite genotyping and marker polymorphism

DNA was extracted from tissue using a standard Chelex protocol (Walsh et al., 1991). All individuals were genotyped at 20 microsatellite loci divided into three multiplexes (Table 1). We used 3 μl of Qiagen Type-it Multiplex PCR Master Mix for the multiplex PCRs (polymerase chain reactions), along with 1 μl of template DNA, and 0.5 μl of primer mix (see Table 1 for concentrations). Total PCR volume was 5.5 μl, and each forward primer was labelled with one of the fluorescent dyes HEX, FAM, NED, ATTO550 or ATTO565. We used the following PCR programme settings: denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, annealing at 55°C (multiplex 1), 54°C (multiplex 2) or 53°C (multiplex 3) for 90 s, extension at 72°C for 30 s and a final extension at 60°C for 30 min. We scored allele sizes against an internal standard (GeneScan 500 LIZ; Applied Biosystems) in an automatic sequencer (3130xL Genetic Analyzer; Applied Biosystems) and GENEMAPPER software (version 3.7; Applied Biosystems).

We estimated population allele frequencies in CERVUS (version 3.0.7; Kalinowski et al., 2007), using a subset of the fish sampled from the quadrat. To reduce the influence of within-group kinship structure, we chose one male (the dominant male, when he was captured) and one random female from each territory (when females were present in the group) for a total of 233 fish. The markers were highly polymorphic with an average of 16.4 alleles per locus and a mean heterozygosity of 0.75, and all markers adhered to Hardy–Weinberg equilibrium (Table 1).

2.4 | Parentage analysis

We used CERVUS to look for the most likely parents of the juveniles and fry in our data set using individuals that had ≥15 loci successfully genotyped (92.2% of all individuals sampled, which was 95.4% of adult females, 99.0% of adult males, 86.4% of juveniles and 100% of fry). We assigned parentage when the assignment was at least 80% confident (based on maximum-likelihood simulations in CERVUS using parameters given in the Supporting Information) and had at most two allelic mismatches. First, we searched for the best matching *pairs* of candidate parents (this yielded 136 matching parent pairs). If no suitable pair was found based on the above criteria, we assessed maternity and paternity in separate analyses. When these analyses identified a matching father and mother for an offspring (but the two putative parents did not match as a pair), parentage was assigned to the single candidate parent with the smaller number of mismatches.

TABLE 1 Marker polymorphism of 20 microsatellites used in this study based on reference population

Locus	*k*	*N*	H_O	H_E	HW *p*-value	Conc. in primer mix (pmol μl^{-1})	Reference
Multiplex 1							
Pmv17	19	233	0.906	0.912	.50	0.5	Crispo et al. (2007)
UNH890	6	232	0.414	0.436	.73	1.0	Carleton et al. (2002)
UNH908	25	235	0.843	0.875	.36	3.0	Carleton et al. (2002)
Gm634	15	234	0.799	0.818	.42	1.0	Lee et al. (2005)
Ppun9	21	233	0.674	0.748	.02	0.5	Taylor et al. (2002)
Hchi59	17	232	0.845	0.864	.52	1.0	Maeda et al. (2008)
UNH216	11	232	0.603	0.584	.83	4.0	Lee and Kocher (1996)
UME002	7	228	0.61	0.627	.44	4.0	Parker and Kornfield (1996)
Multiplex 2							
Pmv3	31	237	0.768	0.775	.04	1.0	Crispo et al. (2007)
GM264	17	234	0.85	0.859	.42	4.0	Lee et al. (2005)
Ppun5	23	233	0.695	0.722	.19	3.0	Taylor et al. (2002)
TmoM13	25	234	0.829	0.907	.44	4.0	Zardoya et al. (1996)
TmoM25	4	231	0.732	0.671	.55	2.0	Zardoya et al. (1996)
Hchi36	4	230	0.539	0.559	.44	1.0	Maeda et al. (2008)
UME003	17	232	0.897	0.869	.56	2.0	Parker and Kornfield (1996)
Multiplex 3							
TmoM11	7	234	0.667	0.677	.87	1.5	Zardoya et al. (1996)
UNH2075	19	233	0.773	0.77	.23	2.5	Albertson et al. (2003)
NP101	18	232	0.81	0.749	.02	3.5	Brandtmann et al. (1999)
Pzeb4	8	232	0.612	0.61	.98	2.0	Van Oppen et al. (1997)
UNH974	33	219	0.863	0.926	.60	4.0	Carleton et al. (2002)

Note: k, number of alleles; *N*, number of individuals genotyped at the particular locus; H_O, observed heterozygosity (proportion of heterozygotes at this locus); H_E, expected heterozygosity (expected proportion of heterozygotes given allele frequencies). HW, adherence to Hardy–Weinberg equilibrium, tested in CERVUS using a Bonferroni correction (Bonferroni-corrected $\alpha = 0.0025$).

If the two candidate parents had the same number of mismatches, but one parent had a confidence of 95%, while the other had 80%, assignment was given to the more confident parent (this occurred three times). Otherwise, no parentage was assigned (this occurred 30 times).

We also searched for the parents of subordinate males. We applied the same assignment criteria as above, except we listed subordinate males as offspring, and females and dominant males as candidate parents. If no suitable parent pair was found, we searched for single paternity matches with dominant males. We did not search for single maternity matches because subordinate males are typically larger than breeding females, which presents ambiguity regarding which fish is the parent and which is the offspring. For this reason, we also did not search for the parents of adult females in our sample.

2.5 | Parentage breakdowns

In total, 353 offspring (juveniles and fry) had enough loci genotyped to be included in the parentage analyses, and we detected at least one parent living in the quadrat for 323 of them (91.5% success rate). Of these offspring, we detected both parents for 136, only the mothers for an additional 114 and only the fathers for an additional 73.

All 73 subordinate males captured had enough loci genotyped to be included in our parentage analysis, and we detected either both parents or just the father living in the quadrat for 48 of them (65.8%). We detected both parents for 23 of these subordinate males, and their fathers (dominant males) for an additional 25.

2.6 | Statistical analyses

All statistical analyses were performed in R (R Core Team, 2020, version 4.0.3). When fitting generalized linear models (GLMs), and linear or generalized linear mixed effects models (LMMs and GLMMs), we used the package "glmmTMB" (Brooks et al., 2017, version 1.0.2.1). All distribution families and link functions in our analyses were determined by inspection of model diagnostics (using the packages "performance," Lüdecke et al., 2020, version 0.6.1, and "Dharma," Hartig, 2020, v. 0.3.3.0) and also chosen for interpretability.

2.6.1 | Are there constraints on the establishment of new territories?

We examined what constraints might limit opportunities for independent breeding from two perspectives; we first asked where in the colony new territories can be established, and then we asked whether males must reach a certain body size before seeking to establish a new territory. Here, we considered new territories to be those held by solitary males, as these were generally in the process of being excavated. We calculated the distances between each territory and the colony centroid and fit these distances with a linear regression model, specifying territory type (two-level categorical: solitary vs. group) as the predictor. We ran 10,000 permutations of this model without replacement, randomizing the territory type label each round. We calculated the *p*-value as the proportion of randomized trials yielding results more extreme than our observed data. Next, we compared the sizes of solitary males to those living in groups. We fit an LMM to male SL (cm, continuous), and included the type of male as a predictor (three-level categorical: solitary male, dominant male in group and subordinate males in group). To account for nonindependence among data points, we included a random intercept of "group ID" and made pairwise comparisons between solitary males and the two types of group-living males.

2.6.2 | When do offspring disperse from their natal groups?

We asked whether there is a particular size/age when offspring disperse from their parents. We fit a GLMM assuming a binomial error distribution and a complementary-log-log link function to a binary outcome variable describing whether offspring were still living with both their parents (suggesting that the offspring had not yet dispersed) or with neither parent (suggesting that they had dispersed). Note, the possibility that offspring may live apart from their parents if both parents had emigrated makes this analysis more conservative. We included offspring SL (cm) as a predictor and "group ID" as a random intercept. This analysis only considered fry and juveniles for which both parents could be detected, and where they were living with either both parents or neither parent ($N = 93$ from 47 groups).

2.6.3 | Does reproductive success increase with adult body size?

We first asked whether dominant male body size correlates with the number of females residing in his territory. We fit a GLM assuming a Poisson error distribution with a log link function to the number of females in the group (count), and included male SL as a predictor (cm). Of 128 groups sampled, seven dominant males evaded capture and were not included in these analyses.

We then asked whether larger adults secure greater proportions of the within-group offspring (juveniles and fry). We fit a GLMM assuming a binomial error distribution and a complementary-log-log link function to the proportion of within-group offspring attributable to each adult in the group. As predictors, we included the sex of the adult (two-level categorical), their relative body size and their two-way interaction term. To calculate relative body size, we divided the SL of each adult by the average SL of all same-sex cohabiting adults per group. Relative body size therefore captures variation in size relative to other potential competitors of the same sex within each individual's group. We included "group ID" as a random intercept. Note that our data here are likely to have negative intracluster correlations, which can lead to deflated Type I error rates, though a large number of clusters as we have here can help to compensate for such biases (Dormann et al., 2013; Nielsen et al., 2021). In the Supporting Information, we present an analogous model yielding qualitatively similar results where the response variable is the absolute number of within-group offspring sired or produced rather than a proportion (see Table S1 and Figure S1).

Furthermore, because the largest male in each group is also the dominant, any effect of male body size might also be captured by male social rank. We therefore repeated the above GLMM, but focused only on males, and we replaced sex as a predictor with "male social rank" (two-level categorical: subordinate vs. dominant).

2.6.4 | How is reproduction shared between dominant, subordinate and other-group individuals?

We examined how offspring paternity was split between dominant males, subordinate males and males currently living in other groups by fitting a multinomial baseline-category logit mixed effects model (MLMM, using the "mblogit" function in the package "mclogit," Elff, 2021, version 0.8.7.3). This model examines how different predictors affect the odds of falling into one response category relative to a baseline category, which we set as the dominant male for this analysis. The response was a categorical variable indicating the sire-type for each offspring (this analysis only considered offspring for which a father could be identified). We included offspring SL (zeroed on 0.4 cm, which was the smallest offspring in our sample) as a predictor to capture variation in time since hatching (i.e., the time duration between offspring hatching and being sampled), which may affect their likelihood of being in the same group as their parents (e.g., due to group switching). We included the number of males in the offspring's group (mean-centred and scaled to have a standard deviation, *SD*, of 1), and the SL of the dominant male (mean-centred and scaled). We included a random intercept of "group ID." We followed this multinomial model with two more targeted GLMMs. The first investigated whether offspring sired by subordinate males become more prevalent as the number of subordinate males in each group increases. Here, we only considered groups in which there were subordinate males (i.e., multi-male groups) and fit a GLMM assuming a binomial error distribution and a cumulative-log-log link function. We fit the paternity of offspring as a binary response variable indicating whether they were sired by the dominant male

or by a subordinate male in their group (note that offspring sired by males living in other groups were not included in this analysis). We included the same predictor variables and random effects structure as described above in the multinomial model. The second follow-up GLMM investigated the proportion of *recently produced* offspring in each group that were sired by dominant males relative to all other sire types, and we did this by examining its intercept term. Since offspring body size was zeroed on its minimum, the intercept reflects the probability that the youngest (smallest) offspring were sired by the dominant male in each group, and the regression line reflects how this probability changes with time since hatching. Here, we fit a GLMM assuming a binomial error distribution and a logit link function to the paternity of each offspring indicating whether they were sired by the dominant male or by another sire type (i.e., subordinate males or males living in a different group). We included the same predictor variables and random effects structure as described above.

Next, we examined how offspring maternity was split between within-group dams and dams living in other groups. We fit a binomial GLMM with a logit link function, and included the maternity of each offspring as a binary response indicating whether they were produced by a within-group or other-group female. As predictors, we included offspring SL (centred on 0.4 cm as above), and the number of females in the offspring's group (mean-centred and scaled). We included a random intercept of "group ID."

The above analyses revealed (i) that most fry and juveniles were sired by current dominant males, and (ii) that as offspring got older, they were not always living together with their genetic parents. These findings led us to conduct two follow-up investigations of the data. First, we considered the possibility that many older offspring had been produced *before* the current dominants were even reproductively capable in their group. Therefore, to obtain an estimate of reproductive skew for each sex, we examined the parentage of two specific subsets of offspring where we could deduce that they had been produced *after* the current dominant individuals had started reproducing in their groups. The first subset, comprising the offspring produced while the current dominant males were reproductively active in their groups, was based on the following criteria: (i) only multi-offspring groups were considered where the dominant male had sired at least one offspring, (ii) only the set of one or more offspring was considered that were *smaller* than the largest offspring of the dominant male and (iii) only offspring that were living in the same group as at least one of their parents were considered (suggesting that these offspring were hatched and raised in the focal group, and had not immigrated from a neighbouring group). We used analogous criteria for establishing the second subset, comprising the offspring produced while the current dominant females were reproductively active in their groups. Here, we included an additional criterion that there be clear size differences among cohabiting females, such that at least one female could be identified as being dominant. To consider as many offspring as possible, we included subordinate males in the sets of offspring when their parentage could be resolved.

Second, we considered the result that offspring were less likely to still be living with their sire or their dam as they got larger and older (here, we used offspring size, SL, as a proxy variable for "time since hatching"). This pattern is likely because as time progresses since the hatching of an offspring, parents and offspring are more likely to move away from one another and join new groups. However, there is an alternate explanation, namely that the odds of offspring living with their sire or their dam can change as a function of historical conditions that were either more or less conducive to extra-group reproduction. Here, *time of hatching* (representing variation in historical conditions) must be accounted for in addition to *time since hatching* (representing variation in the time available for group switching). In our study, both these variables can be decoupled from one another, because sampling took place over the course of approximately 7 weeks. We therefore back-calculated the hatching dates of each offspring in our data set by using a juvenile growth equation derived by Kohler (1998) for *N. multifasciatus* held in the laboratory; SL in mm = 0.19 × age in days +3.42 (see Figure S2). While the relationship between age and size was very strong when measured under laboratory conditions (R^2 = 0.99, Kohler, 1998), we acknowledge that it may be less strong under the influence of environmental factors in the wild. We estimated the date of hatching for each offspring, which ranged from 97 days before the start of our sampling regime to 30 days after the start. We then used a sliding window approach to ask how the odds of offspring living with their sire or their dam changes with their "time since hatching" and "time of hatching." We first controlled for offspring hatch dates ("time of hatching") by looking in time windows with a width of 20 days (e.g., 97–78 days before the start of our sampling). We fit a binary logistic GLMM to the offspring hatched within each time window, fitting male sire type (within-group sire vs. extra-group sire) as the response variable, offspring SL (a proxy for "time since hatching") as a predictor variable and "group ID" as a random intercept. We slid the window in 10-day increments across all hatch dates in our data set. We also repeated this process but fit the models with female dam type (within-group dam vs. extra-group dam) as the response variable. Next, we repeated this approach but used sliding windows to control for offspring size (i.e., "time since hatching"). We fit our models within offspring size windows with a width of 4 mm (starting at 4–7 mm), and included estimated hatch date as a continuous predictor variable. We slid the window in 2-mm increments across the full offspring size range in our data set.

3 | RESULTS

3.1 | Group composition and establishment of new territories

Neolamprologus multifasciatus territories were densely spaced on the shell bed, with only 28.8 ± 11.4 cm (average ± *SD*, range = 6.9–109.1 cm) between nearest neighbouring territories. Territories could be occupied by one individual (solitary males) up to 22 individuals (males, females and juveniles, excluding fry). All territories contained a dominant male (though seven evaded our capture). The average (±*SD*) number of fish per group was 6.3 ± 4.4 with an

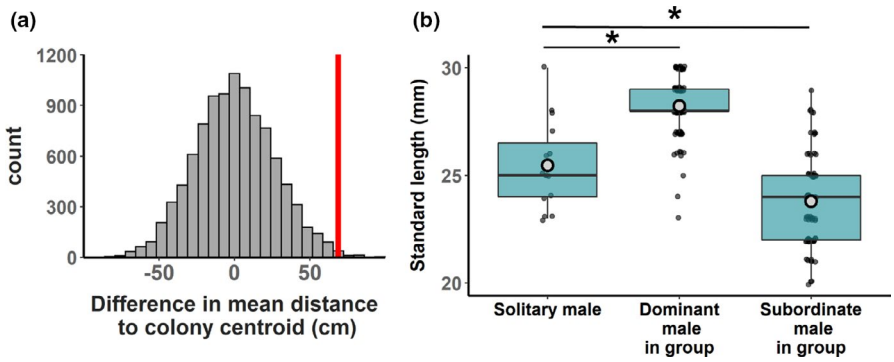

FIGURE 2 (a) Histogram showing the null distribution for the differences in mean distance to the colony centroid between the territories held by solitary males and those held by groups of fish. Null distribution was derived from a permutation test described in the Methods, and the red vertical line indicates our observed difference value. (b) Standard lengths of males according to whether they were solitary, group-living, dominant or subordinate. Box plots indicate sample means (open circles), sample medians (horizontal lines), first and third quartiles (boxes), and the range of data within 1.5 interquartile distances (whiskers). Asterisks (*) indicate significant effects at $p < .05$

adult sex ratio of 1:1.25 (male/female), which are comparable values to those of a field census conducted by Kohler (1998) in a nearby population (7.4 ± 4.4, 1:1.46). Groups contained on average (±*SD*) 1.5 ± 0.9 males (range = 1–5), 1.9 ± 1.4 females (range = 0–6) and 3.0 ± 3.0 juveniles (range = 0–14). Out of 128 territories in our quadrat, 15 were held by solitary males that were in the process of excavating shells from the sand. Solitary males established their territories on the periphery of the colony (Figure 1), on average 68.8 cm further away from the colony centroid than group-held territories (permutation test, $p = .0057$, Figure 2a).

Males and females were on average (±*SD*) 26.3 ± 2.7 mm SL (range = 20–30 mm) and 20.4 ± 0.9 mm SL (range = 18–23 mm) respectively. Juveniles, which are individuals large enough to emerge from their shells, but do not yet have banding patterns on the sides of their bodies, were 16.8 ± 2.9 mm SL (range = 9–23 mm). Based on growth curves established under laboratory settings, an SL of 23 mm corresponds to an age of ~100 days (Kohler, 1998). Fry were 5.6 ± 1.4 mm SL (range = 4–8 mm). On average, solitary males were smaller than dominant males (LMM, est. ± *SE* = −0.28 ± 0.05, $z = -5.80$, $p < .001$), but larger than subordinate males living in groups (est. ± *SE* = 0.17 ± 0.05, $z = 3.43$, $p < .001$, Figure 2b).

3.2 | Breaking down the parentage of juveniles and fry

Of the 209 juveniles and fry for which a sire could be detected, 111 (~53.1%) were sired by the dominant males in their groups, 14 (~6.7%) were sired by subordinate males in their groups and 84 (~40.2%) were sired by males currently living in other groups. Of the 250 juveniles and fry for which a dam could be detected, 187 (~74.8%) were produced by females in their groups and 63 (~25.2%) were produced by females currently living elsewhere. When not in the same group as their parents, offspring were on average 115 cm (range: 12–445 cm) away from their mothers and 88 cm (range: 16–435 cm) away from their fathers (Figure 3a,b). Most juveniles and fry for which both parents were detected ($N = 136$) were still living with them, or just with their mothers (Figure 3c). Out of 93 offspring living either with both or neither of their parents, 75 (~80.6%) were still living with their parents suggesting that they had not yet dispersed, and offspring body size did not significantly correlate with this probability (GLMM, est. ± *SE* = −3.00 ± 2.62, $z = -1.14$, $p = .25$), though there was a visible trend for the largest offspring to be less likely to be living with their parents (Figure S3).

We assume that most of the undetected dams and sires of the offspring in our sample died prior to sampling. This is because we captured nearly every adult fish currently living in the quadrat, and we deem it unlikely that many fish emigrated beyond the quadrat due to their relatively short movement distances on average and the putative risks of crossing the sand barrier surrounding the colony.

3.3 | Breaking down the parentage of subordinate adult males

Of the 23 subordinate males for which both parents were detected, 18 (~78.3%) were living in the same group as at least one of their parents. Of the 48 subordinate males for which a (dominant male) sire was detected, 22 (~45.8%) were living in the same group as him, while the remaining 26 were living separately from their fathers. When not in the same group as their fathers, subordinate males were living on average 98 cm (range: 21–454 cm) away.

3.4 | Larger males are more reproductively successful within groups

We detected a significant positive relationship between the size of the dominant male and the number of females residing with him

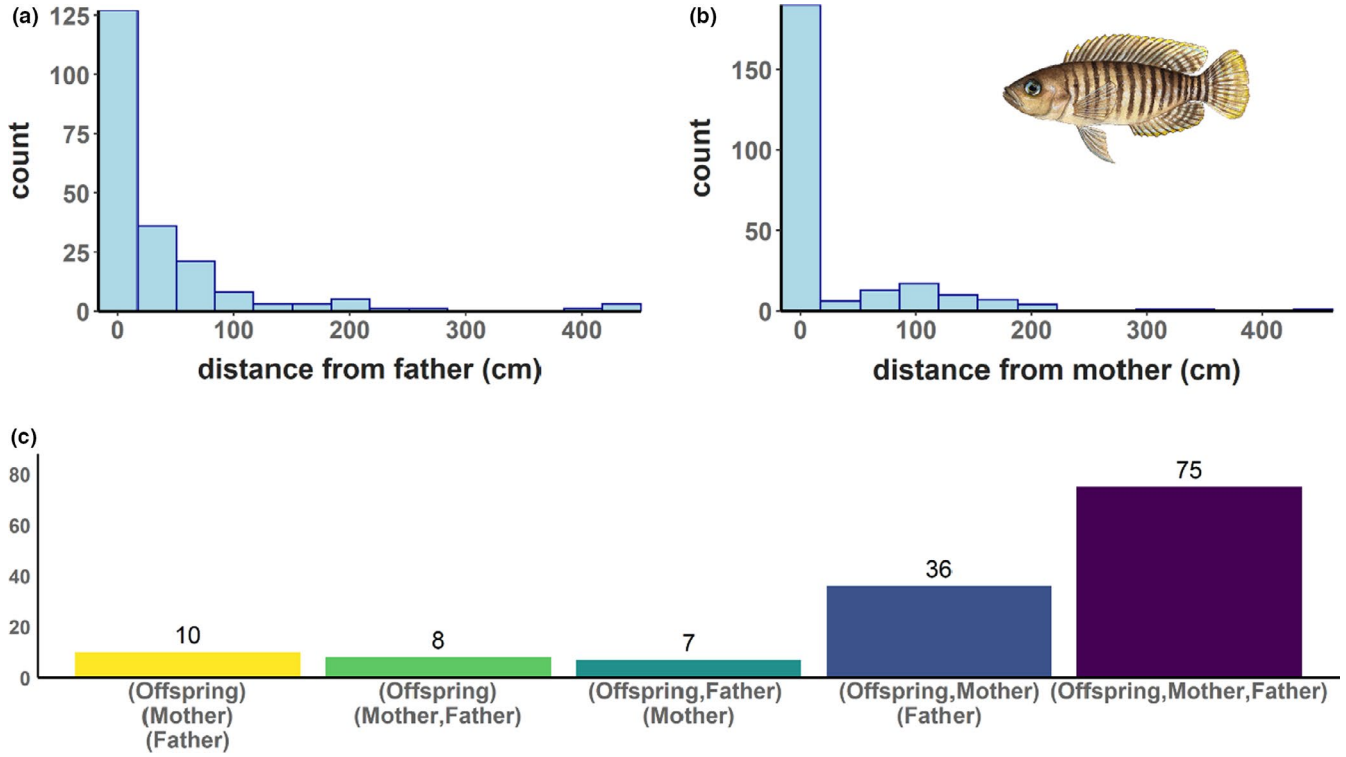

FIGURE 3 (a and b) Histograms illustrating the distances between offspring (juveniles and fry) and their fathers and mothers respectively. (c) Counts of offspring living with or away from their parents. In (c), only the subset of offspring for whom both parents could be detected are shown. Fish living together in the same group are enclosed in the same parentheses. *Neolamprologus multifasciatus* in upper right corner was illustrated by Alexander Viertler

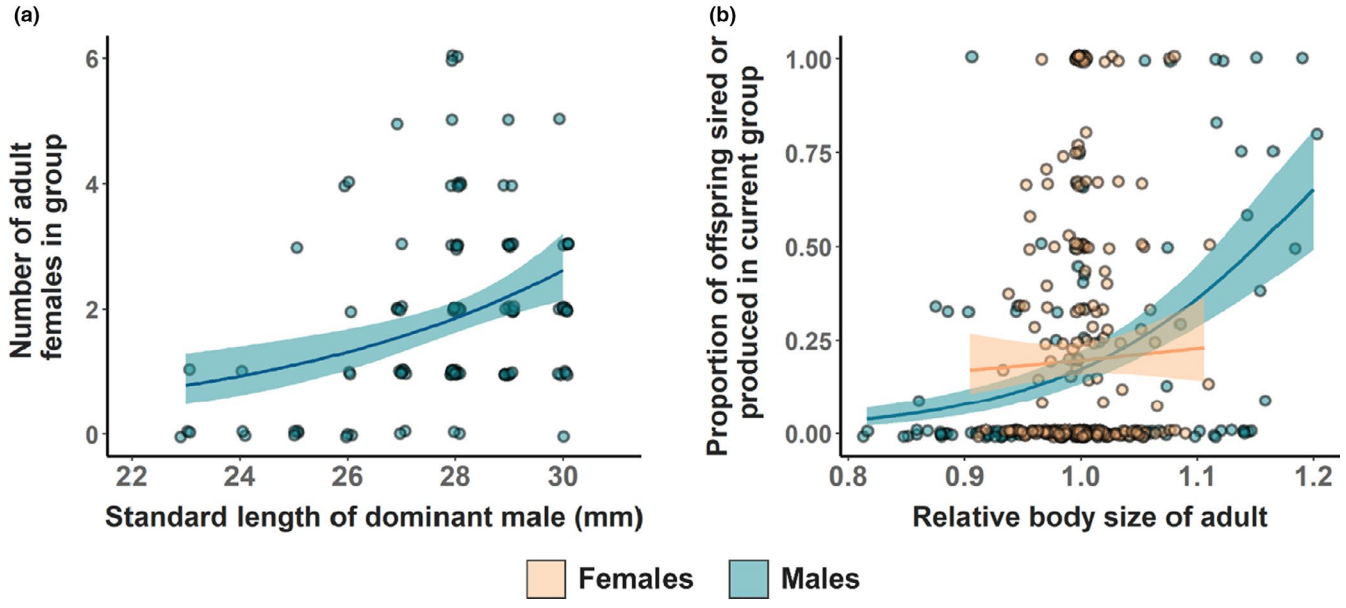

FIGURE 4 (a) The number of females residing in a territory increases with the body size of the dominant male on that territory. (b) The proportion of offspring in a group sired by a within-group male increases with male relative body size, but the proportion of offspring produced by a within-group female does not change with female relative body size. In all panels, predicted fits and 95% confidence intervals from models described in the Methods are shown

(GLM, est. ± *SE* =1.72 ± 0.46, $z = 3.73$, $p = .00019$, Figure 4a). The proportion of within-group offspring sired or produced by each adult depended on their sex and relative body size (GLMM interaction term, Table 2, Figure 4b). Relative body size did not correlate with the proportion of offspring produced by females, but it did in males (Table 2 and Figure 4b). Among males, we detected no significant

TABLE 2 Output from a binomial generalized linear mixed effects model with a cumulative-log-log link function, examining the proportion of within-group offspring sired or produced by within-group adults as a function of the adults' sex and relative body size

Model term	Estimate ± *SE*	*z* value	*p* value
Intercept (female is reference level)	−3.19 ± 2.41	−1.32	.19
Intercept (male is reference level)	−10.21 ± 1.27	−8.04	**<.0001**
Relative body size (female is reference level)	1.67 ± 2.41	0.70	.49
Relative body size (male is reference level)	8.55 ± 1.21	7.08	**<.0001**
Sex (female is reference level)	−7.02 ± 2.72	−2.58	**.010**
Relative body size: Sex (female is reference level)	6.88 ± 2.69	2.56	**.011**

Note: Significant *p* values at $\alpha = 0.05$ are in bold. Note that we present the results of this model for when either sex is treated as the reference level.

interaction between male social rank and relative body size on the proportion of within-group offspring sired ($p = .54$) and so this term was dropped from the final model. Dominants sired a significantly higher proportion of the within-group offspring than subordinate males (GLMM, est. ± *SE* =1.39 ± 0.46, $z = 3.0$, $p = .0028$), and even after accounting for social rank, relatively larger males sired higher proportions of within-group offspring (est. ± *SE* =4.73 ± 2.26, $z = 2.09$, $p = .036$).

3.5 | Offspring more likely to be sired by same-group dominant males than subordinates or males currently living elsewhere

Dominant males were more likely to be the sires of the youngest juveniles and fry in each group than either males living in other groups (MLMM intercept term, Table 3a.1) or subordinate males (Table 3a.2). However, juveniles and fry were more likely to be assigned to a male living in a different group as the offspring got older (i.e., as they grew in SL, Table 3a.1). Approximately 79.8% of these "extra-group" fathers (67 out of 84) were currently dominant males in their own groups. The proportion of offspring sired by dominant males also decreased when subordinate males were in the group (Table 3a.2), but beyond subordinate males being present in the group, the number of subordinates had no detectable effect on the proportion of offspring they sired (GLMM, Table 3b). Overall, dominant males were far more likely than any of the other male types combined (i.e., subordinates or males from other groups) to have sired the youngest offspring in a group (GLMM, intercept term, Table 3c and Figure 5a).

3.6 | Offspring are more likely to be produced by within-group females than females currently living elsewhere

Within-group females were more likely than females living in other groups to have produced the smallest/youngest offspring (GLMM, intercept term, Table 3d and Figure 5b). Offspring were more likely to have been produced by these "extra-group" mothers as they got larger, and less likely to have been produced by them as the number of females within a group increased (Table 3d).

3.7 | Reproductive division while dominants are active in their group: monopolization by dominant males, but sharing among females

The subset of offspring produced *after* dominant males had become reproductively active in their groups comprised 100 offspring (fry, juveniles and subordinate males) from 34 groups. Of these, 76 (76%) were sired by the dominant male, three (3%) by subordinate males, three (3%) by males living in different groups and 18 (18%) by unknown males (Figure 6). The reproductive bias in favour of dominant males was contrasted by the pattern in females. The subset of offspring produced after their same-group dominant female(s) had become reproductively active comprised 62 offspring from 18 groups. Of these, 24 (~38.7%) were produced by the largest, dominant female(s) in the group, 34 (~54.8%) by smaller, subordinate females, and four (~6.5%) by unknown females (Figure 6). Figures S4 and S5 provide more detailed breakdowns of the offspring in these subsets.

3.8 | Older offspring live apart from their genetic parents because of group-switching, not because of historical breeding conditions

While controlling for the dates when offspring were estimated to have hatched, we found that larger/older offspring were more likely to be living apart from their genetic parents (Tables S2 and S3). This was specifically the case for offspring that were at least 15 mm in standard length, and estimated to have hatched at least 58 days prior to the start of our sampling regime. However, while controlling for the amount of time that had elapsed since the hatching of each offspring (i.e., using offspring size as a proxy), we found that the estimated date of hatching per se was not significantly related to the probability of offspring living with their genetic parents (Tables S4 and S5). Together, this suggests that time since hatching, rather than time of hatching, primarily explains whether offspring are living with or apart from their parents.

	Estimate ± *SE*	*z* value	*p* value
(a.1) Sire is male living in another group versus sire is dominant male in same group			
Intercept	−2.23 ± 0.84	−2.67	**.008**
Size of offspring (centred on minimum)	1.36 ± 0.62	2.20	**.028**
Size of dominant male (scaled)	0.17 ± 0.30	0.57	.57
Number of males in group (scaled)	0.06 ± 0.36	0.17	.86
(a.2) Sire is subordinate male versus sire is dominant male			
Intercept	−4.58 ± 1.65	−2.78	**.005**
Size of offspring (centred on minimum)	1.58 ± 1.17	1.35	.18
Size of dominant male (scaled)	−0.22 ± 1.07	−0.21	.84
Number of males in group (scaled)	1.05 ± 0.42	2.53	**.011**
(b) Sire is subordinate male versus sire is dominant male (in multi-male groups)			
Intercept	−4.59 ± 2.21	−2.08	**.038**
Size of offspring (centred on minimum)	1.71 ± 1.54	1.11	.27
Size of dominant male (scaled)	−0.37 ± 0.69	−0.54	.59
Number of males in multi-male group (scaled)	0.50 ± 0.66	0.76	.45
(c) Sire is subordinate male or a male living in another group versus sire is dominant male			
Intercept	−3.88 ± 1.37	−2.84	**.005**
Size of offspring (centred on minimum)	2.65 ± 0.95	2.78	**.005**
Size of dominant male (scaled)	0.26 ± 0.47	0.55	.58
Number of males in multi-male group (scaled)	1.01 ± 0.74	1.36	.17
(d) Dam is within-group female versus dam is living in another group			
Intercept	3.16 ± 0.82	3.85	**.0001**
Size of offspring (centred on minimum)	−1.24 ± 0.57	−2.19	**.028**
Number of females in group (scaled)	0.72 ± 0.32	2.26	**.024**

Note: Significant *p* values at α =0.05 are in bold.

TABLE 3 (a) Output from a multinomial baseline-category logit mixed effects model, examining the probability that an offspring (juvenile or fry) could be assigned to (a.1) the dominant male in their group versus a male currently living elsewhere, and (a.2) the dominant male versus a subordinate male in their group. (b) Output from a follow-up binomial generalized linear mixed effects model assuming a cumulative-log-log link function, considering only multi-male groups, examining the probability that an offspring was sired by the dominant male versus a subordinate male in their group. (c) Output from a follow-up binomial generalized linear mixed effects model assuming a logit link function, examining the probability that an offspring was sired by the dominant male in their group versus any other type of male sire. (d) Output from a binomial generalized linear mixed effects model assuming a logit link function, examining the probability that an offspring was produced by a within-group female versus a female currently living elsewhere

4 | DISCUSSION

4.1 | Ecological constraints limit movement and establishment of new territories

Ecological constraints play an important role in the evolution of group-living by restricting individuals' movement within their habitat and by affecting their options for independent breeding (Emlen, 1995; Krause & Ruxton, 2002). Our data suggest that ecological constraints are strong in the group-living cichlid *Neolamprologus multifasciatus*. Movement distances are exceptionally short, as our parent–offspring pairs were either living together or separated by only ~100 cm on average. The farthest distance separating a parent and their offspring was ~450 cm, which is still notably smaller than the dimensions of our quadrat. This restricted scale of movement is probably a consequence of the dangers of leaving a territory and the shelters therein (Schradin & Lamprecht, 2002). It may also be difficult for subordinate males to switch groups, because fish prospecting to join other groups, especially males, are met with intense resistance from residents (Gübel et al., 2021). This implies that small, subordinate males are not a significant flight risk from their current groups, which has implications for the division of reproduction between dominants and subordinates within groups as we discuss further below (Cant & Johnstone, 2009).

The establishment of a new territory, the precursor of a future breeding group, is always performed by a solitary male (Jordan et al., 2016). Here, we show that these solitary males are relatively large, which we interpret as older subordinates seeking to become dominants in a group of their own. Thus, independent breeding may not become a viable tactic until males reach a large body size, possibly due to the energetic demands of excavating shells and/or their vulnerability to predators (A.B., L.K. and A.J., personal observations). We also found that solitary males were generally restricted to the outskirts of the colony. In numerous species, subordinate individuals can be provoked to disperse and breed independently when vacant breeding space becomes available nearby: for example, Seychelles warbler, *Acrocephalus sechellensis*, Komdeur (1992); *Neolamprologus pulcher*, Bergmüller et al., (2005). On the densely populated shell beds, *N. multifasciatus* may only find vacant space near the periphery of the colony. It would therefore be valuable for future research to investigate the relative roles of ecological constraints and the benefits of philopatry in driving group switching or establishment decisions.

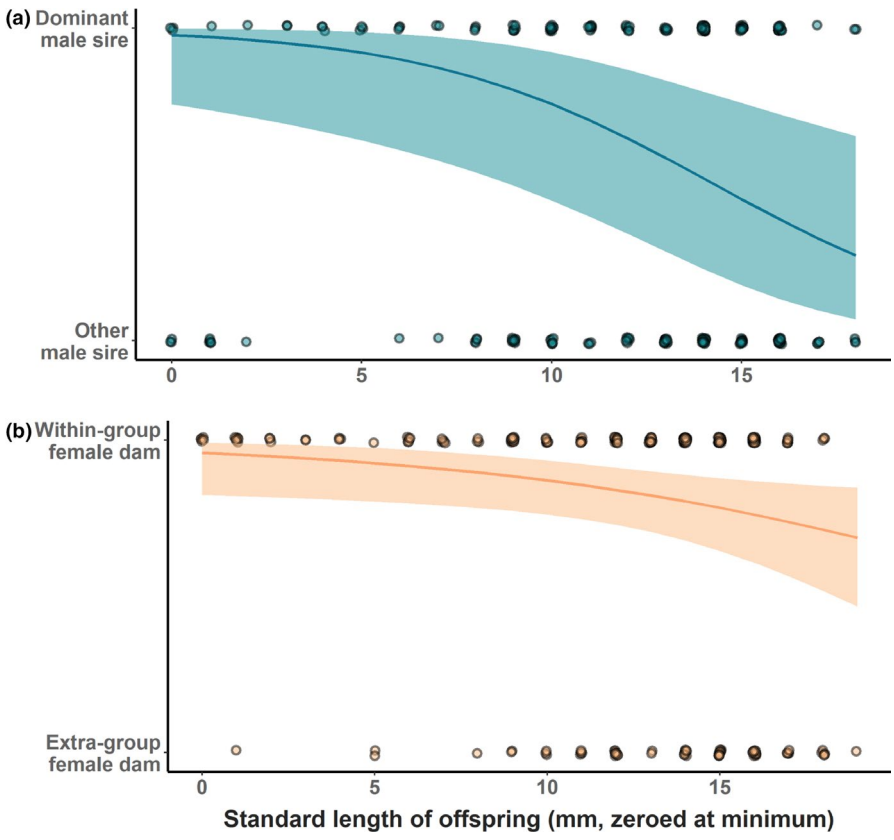

FIGURE 5 (a) Same-group dominant males are highly likely to be the fathers of the smallest (and hence youngest) offspring in their groups (see also Table 3c). (b) Within-group females are also highly likely to have produced the smallest offspring in their groups (see also Table 3d). Both panels show predicted fits and 95% confidence intervals from binomial generalized linear mixed effects models described in Methods

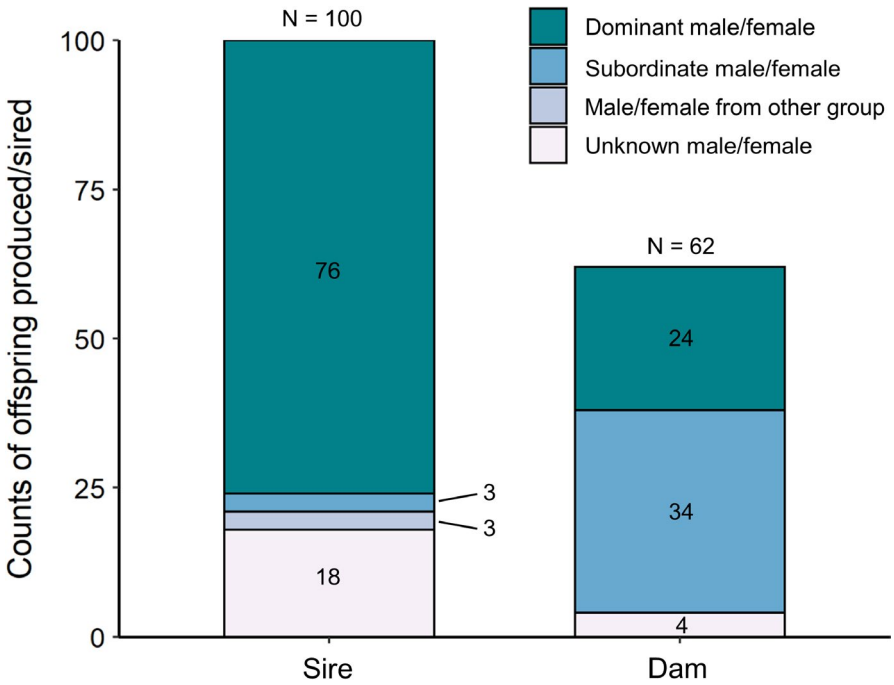

FIGURE 6 Parentage assignments for the subsets of offspring (fry, juveniles and subordinate males) that were produced during the time windows when dominant males and dominant females were reproductively active in their groups (see Methods). Total sample sizes are given above each bar, and the sample size breakdown is given on each bar segment. The plot illustrates the high reproductive skew among males within a group, but low skew among females in *Neolamprologus multifasciatus*

4.2 | Large dominant males live with more females

Larger dominant males possessed territories with more female breeding partners, which may arise from two nonmutually exclusive processes. First, larger dominant males are able to control territories with more shells, which in turn can attract and support more group members (Kohler, 1998). Large body size in many taxa correlates with an individual's resource-holding potential, allowing higher quality resources or territories to be defended (e.g., green frogs, *Rana clamitans*, Wells, 1977; damselflies, *Megaloprepus coerulatus*, Fincke, 1992; *Tropheus* spp., Odreitz & Sefc, 2015; plainfin midshipman fish, *Porichthys notatus*, Bose et al., 2018). Second, larger males may also switch groups, forcefully taking over neighbouring territories containing more females and thereby settle into an ideal

despotic distribution (i.e., Fretwell, 1972). Territory takeovers and male–male aggression over resources have been documented in other Tanganyikan cichlids (e.g., *Neolamprologus pulcher*, Stiver et al., 2006; *Lamprologus callipterus*, Maan & Taborsky, 2008). Since male and female *N. multifasciatus* can change group memberships across their lifetimes (present study; Schradin & Lamprecht, 2000), a combination of both processes probably underlies this pattern.

4.3 | Reproductive sharing and monopolization in *N. multifasciatus* groups

Relative body size was strongly correlated with the proportion of offspring sired by males in their groups, but not with the proportion of offspring produced by females. Small, subordinate males were very rarely the fathers of offspring in their groups (only ~6.7% of the offspring for which we detected a sire could be assigned to a current subordinate male). Since juveniles were sampled up to a size of 2.3 cm SL (~100 days of age), changes in group composition since spawning could confound parentage assignments. Adult group switching, for instance, could be misinterpreted as extra-group reproduction. Our subsequent analyses, accounting for time since spawning, indicated that dominant males were far more likely than any other type of male (i.e., subordinates or males in other groups) to be the fathers of the youngest offspring in their groups. They also sired the vast majority of offspring produced in their groups *after* becoming reproductively active there. In contrast, females of all sizes produced similar proportions of offspring in their groups. This is indicative of high reproductive skew among males, but weak or no skew among females. Our findings confirm that *N. multifasciatus* live in polygynous groups or harems, which had previously only been speculated (Kohler, 1998; Taborsky, 2001).

Numerous social and ecological factors have been presented to explain the extent of reproductive skew, including the potential for inbreeding (Riehl, 2017), seasonal variation in food availability (Nichols et al., 2012), the availability of outside options for independent breeding (Nelson-Flower et al., 2018), and social competition or reproductive suppression (Clutton-Brock et al., 2008). In the southern pied babbler, *Turdoides bicolor*, reproductive skew in groups is higher in males than in females, and it has been suggested that inbreeding avoidance and the ability of subordinates to resist the attempts of dominants to suppress their reproduction explain these patterns in skew (Nelson-Flower et al., 2018). In brown jays, *Cyanocorax morio*, suppression of subordinate female reproduction and female control over paternity interact to result in higher reproductive skew in females than in males (Williams, 2004). As outlined above, subordinate male *N. multifasciatus* have limited outside options for independent reproduction, which may explain high reproductive skew among males: in the absence of a credible threat of group abandonment by subordinates, dominants do not need to concede paternity shares to them to retain the subordinates in the group. Moreover, the struggle for paternity shares may then resemble a tug-of-war scenario (Reeve et al., 1998; Reeve & Shen, 2013) wherein relative resource-holding potential influences the ultimate division of paternity, which will always favour the dominant male. Tolerating subordinate males in a group may offer benefits to dominant males, for example by load lightening of territory maintenance and defence tasks, by offering predator dilution effects or by unearthing shells that can support a greater number of females. Furthermore, relatedness between dominant males and subordinate males may facilitate tolerance, especially if subordinates queue to inherit the territory in the future. Indeed, 22 out of 48 subordinate males for which paternity could be resolved (45.8%) were still living with their fathers. On the other hand, subordinate males may elevate foraging competition among group members, and so future research should establish the specific costs and benefits conferred by subordinate males in *N. multifasciatus* groups.

Female parentage was less skewed than male parentage, and one explanation for this sex difference may be the physical partitioning of the territory. While the dominant male freely traverses the whole territory, females reside in discrete subterritories, which contain shells that they use for reproduction (Bose et al., 2021; Schradin & Lamprecht, 2002). Thus, reproductive suppression among females may be precluded by their distinct patterns of space-use. Female–female aggression, which maintains subterritory boundaries, is also thought to elicit male peacekeeping behaviour. Peacekeeping involves dominant males physically intervening in female–female contests, limiting their escalation (Bose et al., 2021; Gübel et al., 2021; Schradin & Lamprecht, 2000). Thus, we propose that low reproductive skew among *N. multifasciatus* females is supported by the maintenance of physical separation between cohabiting females, while high skew among males is due to dominant males monopolizing access to females in their groups without the need to concede paternity to subordinate males because of the poor outside options for independent reproduction.

4.4 | Little evidence for extra-group reproduction

An appreciable number of offspring were found living separately from their genetic parents, and given the age range of offspring in our parentage analyses this raises the question of whether these cases represent extra-group reproduction, emigration by parents and/or emigration by offspring. We systematically narrowed down these explanations with the following steps. First, focusing on those offspring living with both or neither of their parents, most of them (80.6%) were still living with their parents, and there was a weak trend for larger offspring to be more likely to have dispersed away (Figure S3). This suggests that juveniles wait until they reach a large body size and are on the cusp of maturity before dispersing, and that most individuals delay dispersal until after sexual maturity. This implies that movement of breeders and large juveniles or extra-group reproduction could account for most of our observed cross-group parent–offspring pairings. Second, we also examined the parentage of *all* offspring, and similarly found that the likelihood of parents living separately from their offspring increased

with offspring size/age (Table 3 and Figure 5). This indicates that group switching probably accounts for many of our "extra-group" parentage cases, because extra-group reproduction would not be expected to correlate with offspring size (i.e., our proxy for "time since hatching"; see below for our discussion on accounting for historical conditions that might have been more conducive to cuckoldry). Other group-living cichlids, such as *Julidochromis ornatus* (Awata et al., 2005) and *Neolamprologus pulcher* (Dierkes et al., 2005), display similar decreasing relationships between the size/age of offspring and their relatedness to same-group dominants, which can in part be attributable to the movement of adults among groups, and the replacement of breeders within groups. In *N. multifasciatus*, the movement of adults between groups could be stimulated by takeover events whereby neighbouring individuals oust residents, that must then seek settlement elsewhere, or by individuals founding or joining new groups in an attempt to access better reproductive opportunities. Finally, we asked how often very small offspring had parents living in other groups, which would be suggestive of extra-group reproduction. We found that the vast majority of the youngest offspring were produced or sired by same-group females and dominant males, suggesting that breeders gain little, if any, extra-group reproduction and that subordinates cannot readily offset reproductive shortcomings at home with fertilizations elsewhere. A contrasting pattern is seen in groups of a related species, *N. pulcher*, where most offspring are produced by one dominant female, but paternity can be mixed with dominant males, subordinate males and neighbouring extra-group males acquiring fertilizations (Hellmann et al., 2015). In *N. multifasciatus*, extra-group reproduction, or cuckoldry, appears to be exceedingly rare, but also cannot be ruled out. A post-hoc analysis of body sizes for 36 offspring (from 19 groups) that were living with their mothers but had extra-group fathers (see Figure 3c) revealed that 10 of the offspring were smaller (and therefore probably younger) than their extra-group father's offspring in his current group. This suggests that these males were either successful cuckolders or they held a temporary breeding position in the other group before returning to their current group. In the remaining 26 cases, the fathers either had no offspring in their current groups or had offspring in their current groups that were the same size or smaller than the focal young, which is consistent with the fathers having simply emigrated away to their present positions. Taken together, our data are consistent with (i) delayed dispersal of offspring until nearing or after sexual maturity, (ii) the movement of large juveniles and adults between groups, and (iii) low levels of extra-group reproduction despite living at close proximity to neighbouring groups.

4.5 | Inferring mating patterns from parentage data on a wide age range of offspring

There are several notable challenges associated with making mating pattern inferences based on parentage assignments from offspring of a wide span of ages. Dispersal physically separates offspring from their parents, and the odds of this occurring also increases as offspring age. It is therefore important to sample across a wide-enough area–in our case, the confined colony—to ensure that offspring and parents can both be captured even if they are living separately. Group switching also complicates the task of teasing apart within-group reproduction from extra-group reproduction. This is because emigration between groups can lead parents to be falsely implicated in having engaged in extra-group reproduction just because they are no longer living together with their offspring. We addressed this obstacle by investigating offspring parentage in relation to offspring body size, which reflects the time since their spawning or hatching. A remaining challenge is that the prevalence of different mating tactics, such as cuckoldry, may change over time if, for example, ecological conditions fluctuate (e.g., Sefc et al., 2009). Thus, not only does *time since spawning* need to be accounted for (above), but so too does the *time of spawning*. We addressed this by using a sliding window approach wherein we controlled for either the time of hatching or the time since hatching in separate analyses. We found that the estimated date on which offspring hatched did not significantly predict whether they were living with their genetic parents. Instead, we found that for the very oldest offspring in our data set, the more time that had passed since their hatching, the more likely it was that they were living apart from their parents. This pattern is consistent with the dispersal of large juveniles away from their natal groups as they approach or reach sexual maturity. The fact that offspring hatch dates were not significantly correlated with the likelihood of offspring being found with their genetic parents suggests that rates of extra-group reproduction remained steady (and low) over the period of hatching dates covered by our sampling.

5 | CONCLUSIONS

Although the relative payoffs of reproductive decisions are dictated by ecological and social conditions, it can be difficult to make a priori predictions about how reproduction is likely to be partitioned in group-living animals. Often, close examination of the reproductive distributions for both sexes under natural conditions is required. We sought to do this in *N. multifasciatus*, and our analyses revealed that paternity, but not maternity, is highly skewed within groups. Furthermore, outside of the group, options for independent breeding are scarce and the use of cuckoldry is rare. Our study population lives at extreme densities, further contributing to our understanding of the complex relationship between population density and extra-group reproduction (Mayer & Pasinelli, 2013; Westneat & Sherman, 1997). In this study, we were challenged with overcoming the obstacles associated with making mating system inferences from parentage analyses based on a wide age range of offspring, and we demonstrate that patterns of within-group vs. between-group reproduction can still be elucidated even when circumstances preclude very many of the youngest, dependent offspring from being reliably sampled.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Aneesh P. H. Bose and Alex Jordan conceived the research. Aneesh P. H. Bose and Lukas Koch conducted the field work with assistance from Taylor Banda and Lawrence Makasa. Johanna Dabernig-Heinz, Jacqueline Grimm, Sebastian Lang, and Kristina M. Sefc conducted the genotyping analyses. Funding was secured by Aneesh P. H. Bose, Alex Jordan and Kristina M. Sefc. Statistical analyses were conducted by Aneesh P. H. Bose and Kristina M. Sefc. Aneesh P. H. Bose wrote the manuscript with input from all co-authors.

BENEFITS GENERATED

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. All analyses can be reproduced using the data and R code provided in the Supporting Information.

DATA AVAILABILITY STATEMENT

All analyses can be reproduced using the data and R code provided in the Supporting Information. Data citation: Bose, A. P. H., Dabernig-Heinz, J., Koch, L., Grimm, J., Lang, S., Hegedűs, B., Banda, T., Makasa, L., Jordan, A., Sefc, K. M. (2021). Parentage analysis across age cohorts reveals sex differences in reproductive skew in a group-living cichlid fish, *Neolamprologus multifasciatus*.

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