Is biparental defence driven by territory protection, offspring protection or both?

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In many animal systems, the defence of a territory or nest coincides with the defence of offspring, and it is often unclear whether the defence behaviour exists for the purpose of offspring protection, territory protection or a combination of both. In species with biparental care, the drivers of defence behaviours in males may differ from those in females, particularly if there are sex-specific fitness benefits from the current brood or territory. In this study, we present field experiments aimed at elucidating sex differences in the drivers behind nest defence in a fish species (*Variabilichromis moorii*, Cichlidae) with biparental care. High rates of cuckoldry in this species create asymmetries in brood value between mates, suggesting that increased brood survival may be a weaker driver of male than of female defence effort. We conducted parent and offspring removal experiments and found that following the removal of their mates, single males lost significantly more fry than single females. Some single males lost their territories, while others constricted their space use, suggesting that experimental divorce decreased their success in territory defence. Removal of fry from territories guarded by both parents caused the defence behaviour to become more male biased, that is, males contributed relatively more to defence without any fry present. Our results suggest that, relative to that of females, male defence behaviour is more strongly driven by territory retention than by brood protection. Nevertheless, the presence of males improves parents' abilities to keep territory intruders at bay, and thus probably confers benefits to broods. We conclude that the drivers of defence behaviours differ between the sexes in this species, with defence behaviours aligning well with the traditional definition of parental care for females, but less so for males.

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Parental care has long fascinated behavioural ecologists, inspiring decades of intensive research, and yet it can still be a difficult task to determine which behaviours constitute parental care. According to its formal definition, parental care is any

behavioural parental trait (e.g. oviposition site selection, nest building, provisioning of young, protection of young) or non-behavioural parental trait (e.g. provisioning of gametes, viviparity) 'that enhances the fitness of a parent's offspring and is likely to have originated and/or to be currently maintained for this function' (Smiseth, Kölliker, & Royle, 2012, p. 7–8). This definition of parental care is less concerned with the trait or behaviour itself, and more with the evolutionary origins and current fitness benefits of the trait. Although this definition is conceptually clear, researchers are left with the nontrivial task of distinguishing between traits that originated or are currently maintained for the purpose of increasing offspring fitness versus traits that only incidentally offer these benefits as a by-product of other evolutionary functions (Balshine & Sloman, 2011; Royle, Alonzo, & Moore, 2016; Smiseth et al., 2012). For example, longer male parental care durations in burying

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beetles, *Nicrophorus vespilloides*, may serve to ensure male paternity over future broods, even when their care offers no apparent fitness benefits to current offspring (Hopwood, Moore, Tregenza, & Royle, 2015). In fact, apparent male parental care can evolve in the absence of any fitness benefits to offspring if it provides the male with other benefits such as increased paternity assurance (Kahn, Schwanz, & Kokko, 2013; Kvarnemo, 2006). These examples put formal definitions of parental care to the test by highlighting the complex and sometimes confusing task of characterizing parental behaviours. Territorial behaviours can be particularly ambiguous, as defence of nests or territories can benefit offspring raised at these sites, but at the same time defence behaviours may be expressed independently of interest in the current brood (Smiseth et al., 2012; Whiteman & Côté, 2004).

Further complexity arises in biparental species in which both parents cooperate to raise their offspring and to defend a territory or nesting site. In these systems, the evolutionary drivers of defence behaviours may differ between the sexes, and it is often not obvious to what extent each parent's defence is driven by the benefits of offspring protection, territory protection or both. This is especially true when the fitness value of the same brood of offspring differs between the two parents, which occurs frequently in species with extrapair copulations. As with other potentially pleiotropic behaviours, careful experiments are required to tease apart the drivers of defence behaviours, because evidence for one mechanism (e.g. offspring protection) does not preclude the importance of the other (e.g. territory protection).

Here, we probe the drivers of defence behaviour performed by paired, male and female parents in a socially monogamous cichlid fish that is commonly classified as a biparental breeder (Sefc, 2011). Using behavioural experiments, we address factors that are likely to help maintain the parental behaviour and use the term 'driver' in this restricted sense, that is, without respect to the evolutionary origin of the behaviour. In many fishes, broods are raised within established territories and defence against territory intruders is considered the primary form of parental care (Balshine & Sloman, 2011; Smith & Wootton, 1995). In such cases, defence offers multiple benefits by warding off both potential offspring predators and potential territory competitors or exploiters (e.g. Zimmermann et al., 2019). Since territory ownership often supports greater reproductive potential than that gained through alternative reproductive tactics (e.g. Bose, Zimmermann, Henshaw, Fritzsche, & Sefc, 2018; Cogliati et al., 2014), the benefits of territory retention could feasibly explain territory defence behaviour independent of whether current offspring require protection. One way of probing the underlying drivers for defence behaviours and whether they differ between the two parents in biparental systems is to conduct parent and offspring removal experiments. First, by removing one of the two parents, we can test whether the remaining parent compensates for the loss of its partner and successfully retains their brood when burdened with the increased costs of defending alone. If the two parents in a biparental system are similar in their defence capabilities and are equally interested in keeping offspring predators at bay, then offspring survival should be independent of the sex of the remaining parent. Second, we can test whether the division of defence labour between the two parents changes when dependent offspring are removed from their care. Here, if one parent is more invested in protecting offspring than the other parent, then they should take on a larger proportion of the defence labour when offspring are present than when they are removed.

In the current study, we used *Variabilichromis moorii*, a territorial, substrate-spawning cichlid endemic to Lake Tanganyika in East Africa (Karino, 1997; Rossiter, 1991). Both paired and solitary adult *V. moorii* individuals hold rocky territories in the shallow littoral zone, in which they feed, shelter and breed (Karino, 1998; Ota, Hori, & Kohda, 2012; Zimmermann et al., 2019). The territories range in size from 1 to 4 m^2 and can reach densities of up to 1 territory per 3 m^2 (Karino, 1998; Sturmbauer et al., 2008). Competition for territories is strong and associated with frequent agonistic interactions between conspecifics of both sexes (Karino, 1998; Ota et al., 2012; Sturmbauer et al., 2008; Zimmermann et al., 2019). Many nonterritorial adults are also present in the population, and nonterritorial males commonly act as cuckolders when pair-bonded fish spawn on their territories (Bose et al., 2018). There are no data on the process of pair formation, territory establishment and divorce rates, but we have observed that pairs can remain together on the same territory for successive brood cycles (Zimmermann, Fritzsche, & Henshaw, 2017). *Variabilichromis moorii* is sexually monomorphic in size (8–10 cm standard length) and coloration (uniform dark body and fins, with light blue fin edges) and pair bonds form between closely size-matched males and females, suggesting little physical inequality between parents. Spawning takes place year-round. Fry remain in their parents' territory for approximately 100 days (Rossiter, 1991), and both breeders defend vigilantly against territory intrusions (Karino, 1997; Ota et al., 2012; Sturmbauer et al., 2008; Zimmermann et al., 2019). The majority of intruders are either conspecifics or heterospecifics in competition for food (algae/zooplankton) and territories, whereas a small proportion of the intruders are egg and fry predators or piscivorous species (Zimmermann et al., 2019). Defence against territory competitors (i.e. conspecific and heterospecific intruders that compete with *V. moorii* for food and space) is shared equally between male and female partners, while defence against brood predators (i.e. piscivorous fish and egg predators) is performed mainly by females (Zimmermann et al., 2019). Despite the difference in defence frequencies, both males and females were observed to drive away potential brood predators including large individuals (20 cm) of piscivorous species such as *Lepidiolamprologus* spp. (personal observations by H. Zimmermann, made during the field work of Zimmermann et al., 2019), suggesting that both sexes are physically capable of effective brood defence.

The males have unusually low paternity in their nests, losing on average 25%–50% of the offspring produced by their female partners to cuckolders (Bose et al., 2018; Zimmermann et al., 2019). A previous study found no relationship between male share in defence and paternity despite paternity of the paired males varying between 0% and 100% across nests, suggesting that chronic low paternity over evolutionary time has dampened male paternal investment (Zimmermann et al., 2019). Despite rampant cuckoldry, however, territory-holding males achieve higher reproductive success within a brood cycle than nonterritorial cuckolders, and most cuckoldry is committed by unpaired males (Bose et al., 2018). In contrast, females are almost always the genetic mothers of offspring on their territories (Sefc, Mattersdorfer, Sturmbauer, & Koblmüller, 2008; Bose et al., 2018, 2019). The low paternity share in broods produced by their paired mates combined with the possibility to reproduce as cuckolders can lead to asymmetry in brood value between male and female parents (i.e. the extent to which the brood contributes to each parent's fitness). We therefore hypothesized that territory protection is a stronger incentive than brood protection for defence behaviour in male *V. moorii* compared to females. We predicted that mate removals would result in higher fry losses under the care of single males than single females in consequence of their different involvement with brood defence (Zimmermann et al., 2019). We also predicted that fry removals would impact the sexes differently and shift the division of defence labour, which is female biased in the presence of fry (Zimmermann et al., 2019). While females would transition from defending a territory with a high-maternity brood on it to defending just the

territory, males should be less affected, if their defence is mainly motivated by territory protection even while fry are present.

We also examined the ways in which uniparental brood care differed from biparental brood care in two additional field experiments. First, we monitored the space use of experimentally divorced males to test whether males remained closer to the brood after divorce and thereby abandoned their typical position on the territory border. It is not uncommon in biparental cichlids for the female to remain closer to the brood on the territory, while the male keeps a greater distance (e.g. *Neolamprologus toae*, Nakano & Nagoshi, 1990; *Cichlasoma citrinellum*, Rogers, 1988; personal observations by H. Zimmermann, made during the field work of Zimmermann et al., 2019). Studies in the biparental cichlid *Amatitlania nigrofasciata* have demonstrated that these parental roles can be flexibly adjusted when one parent drops out (Itzkowitz, 1984; Itzkowitz, Santangelo, & Richter, 2001; Lavery & Reebs, 1994). We predicted that if *V. moorii* males were motivated to maximize brood survival, then experimentally divorced males would move closer to their brood for more effective protection.

Second, we tested whether male defence, even if not primarily driven by brood protection, contributes to the overall quality of brood defence, that is, the safety provided to the fry. We estimated the quality of brood defence by quantifying the distances that predators/competitors were allowed to intrude into a territory before being attacked and driven away. This distance often covaries with the threat level that the intruder poses to any offspring that are present (Nakano & Nagoshi, 1990; Perrone, 1978; Wisenden, 1994). Frequent closer approaches of predators to the brood are likely to increase predation risk and hence represent a poorer

quality of brood care (Lehtonen, Wong, Svensson, & Meyer, 2011; Sowersby, Lehtonen, & Wong, 2018). Thus, we used defence distance as a proxy for long-term brood care quality, as it was not possible, logistically, to monitor brood survival over the 3-month brood care period of *V. moorii*. We therefore compared the radii of brood defence maintained by biparental pairs with those of experimentally divorced females. A reduction in defence radius after male removal would suggest that fry under solitary female care experience lower quality care than fry under biparental care.

METHODS

Fieldwork took place at the eastern shore of Mutondwe Island, Zambia (8°42′29.4″S 31°07′18.0″E) over 3 years (four field seasons: November 2015, October – November 2017, April 2018 and September – October 2018). This field site hosts a large breeding population of *V. moorii* at depths of 1–8 m in a ca. 2000 m^2 quadrat of relatively homogeneous rocky substratum scattered with small stretches of sand or gravel. We used SCUBA to observe and manipulate territories of breeding *V. moorii* pairs in the context of four different experiments (detailed below). We chose territories that contained a male - female pair of adults as well as free-swimming fry. Information (depth, brood sizes, fry sizes) about all territories used in the experiments is given in Appendix Table A1. All behavioural observations were carried out between 0900 and 1200 hours to minimize diurnal effects (Sturmbauer et al., 2008). All live scoring of fish behaviour was done at approximately 2 m from the territory to minimize disturbance to the residents. Fig. 1 illustrates the timelines of the experiments.
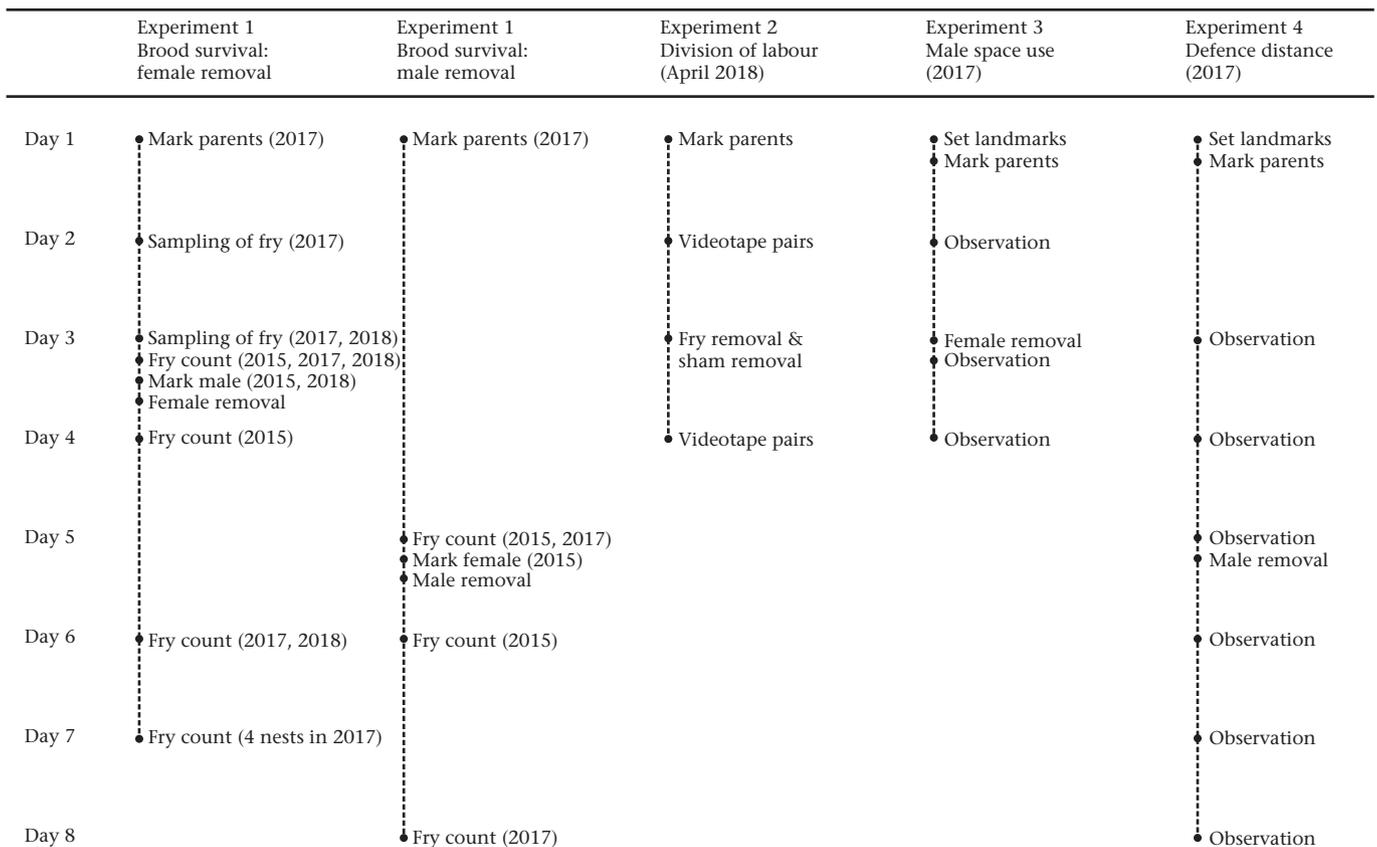
	Experiment 1 Brood survival: female removal	Experiment 1 Brood survival: male removal	Experiment 2 Division of labour (April 2018)	Experiment 3 Male space use (2017)	Experiment 4 Defence distance (2017)
Day 1	Mark parents (2017)	Mark parents (2017)	Mark parents	Set landmarks Mark parents	Set landmarks Mark parents
Day 2	Sampling of fry (2017)		Videotape pairs	Observation	
Day 3	Sampling of fry (2017, 2018) Fry count (2015, 2017, 2018) Mark male (2015, 2018) Female removal		Fry removal & sham removal	Female removal Observation	Observation
Day 4	Fry count (2015)		Videotape pairs	Observation	Observation
Day 5		Fry count (2015, 2017) Mark female (2015) Male removal			Observation Male removal
Day 6	Fry count (2017, 2018)	Fry count (2015)			Observation
Day 7	Fry count (4 nests in 2017)				Observation
Day 8		Fry count (2017)			Observation

Figure 1. Timelines of the experiments. 'Day 1' signifies the first day on which a manipulation took place, but the dates on which the trials were performed vary within experiments. In experiment 1, the schedule of when parents were marked, and when fry were sampled and counted, varied among trials and years (years are in parentheses) due to logistic constraints. Territories used in experiments 3 and 4 are the same as those used in 2017 for experiment 1 (male and female removals).

Experiment 1: Brood Survival after Parent Removal

To test whether males and females perform differently under uniparental conditions, we temporarily removed one parent per territory and monitored brood size and the presence of the remaining parent on the territory over the subsequent days of uniparental brood care. Thirty-three female removal and 19 male removal trials were carried out across four field seasons (female removals: five territories in 2015, 14 territories in 2017, eight territories in April 2018 and six territories in September 2018; male removals: five territories in 2015, 14 territories in 2017).

We used gill nets (mesh width 0.5 cm) to catch the two parental individuals at each territory, sexed them by examining their urogenital papillae, measured their total body length (mm), and marked them according to their sex by clipping their caudal fins (males were clipped along the dorsal edge of the caudal fin, while females were clipped along their ventral edge; fin clips were roughly 12 mm^2 in size). The fin clips were stored in 99.9% ethanol for later genetic parentage analysis.

Next, brood sizes were estimated using either of two approaches: (1) one SCUBA diver made at least four replicate visual counts of the fry while positioned next to the territory (this method was used in 2015) or (2) the full brood was collected in transparent plastic bags, photographed three times, and then returned to the territory (except for certain territories, where a portion of the brood was sampled for genetic parentage analysis, see below). Collecting the fry was facilitated by the fact that free-swimming fry of *V. moorii* tend to school together. We manually guided the swarm of fry into the collection receptacle. To ensure that we captured the complete brood, we ran our fingers through the soft sediment to find leftover fry, which were then moved carefully into the plastic bag one by one. This technique is highly effective at collecting the entire brood of free-swimming fry when performed by trained divers, and the whole procedure lasts approximately 2 min. Fry were counted from each of the three photographs (this method was used in 2017 and 2018). In both approaches, the highest number obtained in the replicate counts was scored as the brood size estimate. After brood sizes were determined and the brood was returned to the territory, one parent was removed from the territory. The remaining parent resumed normal behaviour within a few minutes. During this period the scuba diver ensured the safety of the fry. Fry sizes were determined only for those fry that were collected for genetic parentage analysis (see below) and ranged from 6 to 31 mm body length (Appendix Table A1; unmeasured fry also fell within this size range). The period between marking the parents and mate removal varied between territories (Fig. 1). In 2017, the same territories contributed to this experiment and to experiments 3 and 4. In 2017, parents were observed for 2–4 days prior to fry counting, whereas in 2015 and 2018, marking parents, counting fry and mate removals took place on the same day (Fig. 1). Adult fish that were temporarily removed from their territories were either kept in underwater mesh cages (23 x 23 cm and 55 cm high) ca. 200 m from the study site (in 2015) for 1 day or in concrete tanks (dimensions: 100–200 × 90 cm and 100 cm high) at a nearby facility for up to 3 months. Maximum transport times were ca. 30 min. Some of the removed fish were euthanized for use in another study (Bose et al., 2020). Those fish that were returned to the study quadrat after the experiment resumed territorial behaviour within the quadrat. One to four days after mate removal, brood sizes were again determined using the approaches described above to estimate fry loss under uniparental care. The duration of uniparental care (i.e. the length of the interval between parent removal and the subsequent assessment of brood size) varied across field seasons: 24 h in 2015, and 72 h in 2017 and 2018, excluding four territories in 2017 for which the duration was 96 h (Appendix Table A1, Fig. 1).

Variation among trials in experimental details was due to logistical constraints and was accounted for in the statistical analysis.

From certain territories used in the female removal experiment (from 21 broods in total: seven in 2017, eight in April 2018 and six in September 2018), we also collected ca. 50% of the brood (for parentage analysis) during brood size estimation (see below and Fig. 1). Collected fry were brought to the surface and euthanized in a bath containing MS-222 (1 mg/litre lake water) and preserved in 99.9% ethanol for DNA analysis. We only removed fry from large broods to ensure that we gathered sufficient numbers of offspring with which to estimate brood paternity, but also to ensure that the remaining brood sizes were not unnaturally small. Sampling fry in such a manner had the additional effect of reducing variance in brood sizes among the territories in our sample at the start of the experimental manipulations (brood sizes are reported in Appendix Table A1). Since brood size manipulations, particularly reductions, have the potential to influence patterns of parental investment (e.g. sometimes promoting parental desertion as in the cichlid fish *Aequidens coeruleopunctatus*, Jennions & Polakow, 2001), we confirmed that our partial brood sampling had no detectable effect on subsequent brood losses (see below).

We performed genetic parentage analyses on the subset of 21 broods that were sampled from the female removal trials described above. Microsatellite genotyping of parents and broods was carried out as described by Bose et al. (2018). In brief, we used a standard Chelex protocol to extract DNA from tissue samples (Walsh, Metzger, & Higuchi, 1991). We genotyped the tissue samples from adults and fry with a set of nine polymorphic microsatellite markers (Pmv17, Crispo, Hagen, Glenn, Geneau, & Chapman, 2007; TmoM11, Zardoya et al., 1996; Pzeb3, van Oppen, Rico, Deutsch, Turner, & Hewitt, 1997; UNH 2075, Albertson, Streelman, & Kocher, 2003; Ppun9, Ppun20, Ppun21, Taylor et al., 2002; Hchi59, Hchi94, Maeda et al., 2008). Parentage analyses were performed with the help of the program COLONY (v. 2.0.6.1, Jones & Wang, 2010) as described in detail in Bose et al. (2018). Population allele frequencies were estimated from population samples, which were collected during the same field season as the broods and included both the parents from the investigated territories and additional adult *V. moorii* captured within the study quadrat (2017: $N = 77$; April 2018: $N = 89$; September 2018: $N = 157$). Based on the full-sib group reconstructions of COLONY, fry were identified as within-pair or extrapair offspring and paternity shares of territory-holding males were calculated by dividing the number of within-pair fry by the total number of fry in the territory.

All statistical analyses were conducted in R v. 3.4.4 (R Development Core Team 2018). We first performed a *t* test to check for differences between the total length of experimentally divorced males and females. We next tested whether sampling fry (prior to female removal, see above) subsequently affected brood loss under male-only care. We fitted a generalized linear mixed-effects model (GLMM, R package MASS; Venables & Ripley, 2002) with a quasibinomial error distribution to account for overdispersion of data. We included the proportion of fry lost during the uniparental care interval (i.e. using counts for 'fry retained' and 'fry lost') as our response variable ('fry loss'). We included 'fry sampling' (yes/no) as predictor variable, 'male total body length' (mean-centred and scaled by dividing by the standard deviation) and 'brood size' before mate removal as covariates. We also included the time from mate removal until the postremoval fry count ('duration of uniparental care', in h, scaled by dividing by the root mean square) as an additional covariate to account for the variation across trials in time between fry removal and follow-up checks. The model also included 'date of mate removal' nested within 'season' (2015, 2017, April 2018, September 2018) as a random intercept to account for

temporal nonindependence of observations that were made on the same date.

Then, we tested whether the rate of fry loss differed between the single males and females by fitting a GLMM with a quasibinomial error distribution. We included 'fry loss' as the response variable, the 'sex' of the remaining caregiver as a predictor variable as well as their mean-centred and scaled 'total body length', 'brood size' and 'duration of uniparental care' as covariates. We also fitted 'date of mate removal' nested in 'season' as a random intercept.

Since some parental fish disappeared from their territory after the removal of their partner, we tested for a sex difference in the rate of territory retention between the experimentally divorced males and females. We applied a Fisher's exact test to a contingency table crossing 'sex' with 'territory retention'. Here, we only considered territories where the follow-up checks occurred after 3 days ($N = 24$ male-only and 14 female-only territories) or four days ($N = 4$ male-only territories) since parent removal. We then compared the fry losses between males and females in a data set restricted to single parents that had remained in their territory until the end of the 3- or 4-day observation period ($N = 21$ male-only territories and 14 female-only territories). First, we used a Fisher's exact test to compare the proportions of males and females that had lost their entire broods (i.e. suffered 100% fry loss), and then also fitted a GLMM to compare rates of fry loss between males and females as described above. This data set included trials from three field seasons only (excluding the territories from 2015 which were observed for a shorter duration), which precluded the inclusion of 'season' as random factor, and we only included 'date of mate removal' as a random intercept here.

Finally, we investigated whether a male's success in brood defence was correlated with his share of paternity ($N = 21$ territories with paternity data). We again fitted a GLMM assuming a quasibinomial error distribution and included the proportion of fry lost during his (uni)paternal care phase as the response variable ('fry loss'). 'Paternity share', 'total body length' (mean-centred and scaled), 'brood size' and 'duration of uniparental brood care' (scaled) were included as predictor variables. As above, the model included 'date of mate removal' nested within 'season' as a random intercept.

Experiment 2: Division of Defence Changes after Brood Removal

Here, we tested whether the presence of fry on the territory influences the division of defence labour between males and females. In April 2018, we haphazardly chose 28 breeding pairs within the study quadrat, and randomly assigned them to a 'brood removal' group ($N = 14$) or a control group ($N = 14$). We chose nests with rather young fry (approximately within the first half of their brood care period, 5–15 mm body length). Fry size was measured only in the treatment group (Appendix Table A1), but the fry size distribution in the control group was matched based on visual size estimates. In each group, we carefully captured the two parental individuals, sexed them by inspecting the urogenital papillae, measured their total lengths (mm), and marked the sexes by fin clipping their caudal fins (as above; for timeline, see Fig. 1). The following day, we took a 15 min video (Olympus Tough TG5 underwater digital camera) of each pair to monitor their defence behaviours. It has previously been established that *V. moorii* parents resume brood care and territory defence within minutes after handling and marking (Zimmermann et al., 2019), such that after a 24 h period, the parents are expected to have returned to normal behaviour. On the third day, we captured all fry from the territories in the brood removal group as described for experiment 1. The fry were transported to the surface, euthanized in MS-222 (1 mg/litre lake water) and preserved in 99.9% ethanol for use in other studies.

In the control group, fry were captured in the same way and then carefully released back to the territory after 30 s to control for territory disturbance. Twenty-four hours after these manipulations, we again videotaped the pairs in their territories for 15 min. From each video, the same observer scored all overt and display aggression that was clearly directed towards an intruder for the male and female of each pair separately. Display behaviours consisted of frontal displays (the focal individual approaches another fish with erect gill covers) and lateral displays (the focal individual moves slowly towards another fish showing the lateral side with erect fins). Overt aggression typically consisted of darting towards and biting conspecific and heterospecific intruders. A previous study by Zimmermann et al. (2019) showed that each unfamiliar (i.e. non-neighbouring) fish that approaches a territory of *V. moorii* typically elicits a single defensive response (e.g. an act of aggression) by one of the two territory holders. The number of defence behaviours jointly performed by the pair therefore reflects the intrusion pressure experienced by the territory. This amounted to as much as 5.5 defensive actions/min in the previous study on the same quadrat (Zimmermann et al., 2019). Hence, we expected 15 min observations to accurately capture variation in intrusion pressure and defence behaviour across territories.

We first tested whether the number of defence behaviours performed jointly by pairs differed before and after brood removal (or sham brood removal), using a paired Wilcoxon signed rank test. Next, we fitted a GLMM (R package lme4; Bates, Mächler, Bolker, & Walker, 2015) with a binomial error distribution to test for changes in the division of labour between the parents before and after manipulation. We included the proportion of the joint defences of the pair that was performed by the female as the response variable 'female share of defence' (i.e. using counts for 'female defence behaviours' and 'male defence behaviours' for each 15 min period). We included 'time of observation', a variable indicating whether the observation was made before or after manipulation, as a two-level categorical predictor variable along with 'treatment group' (brood removal versus control) as an additional categorical predictor variable, as well as their interaction term. Since body sizes of paired males and females were strongly correlated (Pearson correlation: $r = 0.46$, $t_{54} = 3.8$, $P = 0.0003$), we included the 'average body size' of the partners (scaled by dividing by the root mean square) as an additional covariate. We fitted 'territory ID' and 'date of first observation' as random intercepts to account for repeated measurements on the same territories and nonindependence of temporally clustered observations. We calculated the intercepts for each treatment group by running the same model twice, once with the control group as reference level and once with the brood removal group as reference.

Experiment 3: Male Behaviour after Female Removal

Next, we tested whether removal of the female affected male behaviour on the territory, specifically their distance to the brood and the extent of utilized space within their territory. A subset of the territories used in the female removal trials from experiment 1 was used here ($N = 14$ territories in 2017, see Appendix Table A1). We captured, sexed, measured and clipped the parental individuals as described in experiment 1. To monitor the position of males relative to their broods, we laid out a star-shaped grid of small painted stones. Starting from the centre of the space occupied by the group of fry, we placed the landmark stones along eight compass-bearing vectors (i.e. N, NE, E, SE, S, SW, W, NW) at intervals of 20 cm, covering a radius of 100 cm around the brood. On the next day (day 2; Fig. 1) while females were still present, the males' positions were monitored for 10 min each. On the third day, the females were removed from the territories. The males' positions

were monitored 1 h after female removal for 10 min and then once more after an additional 24 h for 5 min (Fig. 1). Monitoring of the males' positions took place as follows. Every 10 s during each observation, the same scuba diver (H.Z.) recorded the position of the experimentally divorced males according to the nearest landmark stone (e.g. 60 cm SW). Males never left the grid. Although broods of young *V. moorii* fry tend to stay close together and remain relatively immobile, in four territories the broods shifted their positions after female removal (such that they were no longer located at the 'origin' on the cartesian grid of marker stones). In these cases, the males' distances to their broods were calculated using the new brood positions. Finally, as distances did not differ significantly between days 3 and 4 of the experiment (mean ± SD: day 3 = 15.4 ± 7.0 cm, day 4 = 13.0 ± 7.8 cm; Wilcoxon rank sum test: $W = 61$, $P = 0.43$), data from the two scan samplings following female removal were pooled for analysis.

We fitted a linear mixed-effects model (LMM, R package lme4; Bates et al., 2015) to compare the males' distances from their broods before and after female removal. Sample sizes before and after female removal differed because some males lost their broods (before: 14 males; after: six males). We included the average male distance from the brood as the response variable and 'pair status' as a predictor variable, which indicated whether the distance measures were obtained before or after removal of the female. We included 'territory ID' as a random intercept. The small sample size following the males' brood losses precluded the inclusion of covariates such as brood size or male body size.

Next, we tested for changes in the extent of space used by males after female removal ($N = 10$ males that retained their territory). To place the position data into a coordinate system with brood at the origin, we converted the cardinal directions into radians (angle of the cardinal direction multiplied by π and divided by 180) and multiplied the sine and cosine of the radians by the distance value to obtain x- and y-coordinates, respectively. We used x-y coordinates obtained for each male every 10 s and the function 'kernelUD' (R package 'adehabitatHR'; Calenge, 2006) to calculate areas of utilized space for each male. This function uses kernel estimations to create a probability distribution (Silverman, 1998; Wand & Jones, 1994), i.e. a 'utilization distribution' (UD; Winkle, 1975), giving the probability density that an animal is found at a given point in space. We used this function with the 'href' smoothing parameter to estimate a core area (50% UD) and a total area (99.9% UD) for each male. Based on the UD probability densities, we calculated the core and total areas using the function 'getverticeshr' from the same R package. We fitted two LMMs (R package lme4; Bates et al., 2015), one with the total and one with the core areas as the response variable and included 'pair status' as the predictor variable. We also included 'territory ID' as a random intercept in both models.

Experiment 4: Reduction in Defence Distances after Male Removal

Finally, we tested whether the radius at which females attack territory intruders changes when the male is removed from the territory. Similar to experiment 3, we marked and measured the parental fish and furnished territories with a grid of landmark stones ($N = 14$ of the territories used in the male removal trials of experiment 1 in 2017; see Appendix Table A1). The stones were laid in a star-shaped grid (see Methods, experiment 3 for a detailed description) at 20 cm intervals beginning at 30 cm up to 150 cm from the centre of the space occupied by the brood. We started observations 48 h after these preparations. One scuba diver (H.Z.) observed each nest for 10 min on each of 3 consecutive days with males and females present. Immediately after the third observation session (day 5 of the experiment, Fig. 1), the males were removed

from their territories. On the following 3 days (days 6–8 of the experiment, Fig. 1), the nests were again observed for 10 min per day. During observations, all display and overt aggressive behaviours (as described in experiment 2) directed against conspecific and heterospecific fish by each individual of the territory-holding pair (before male removal) and by the solitary females (after male removal) were scored and the distance from the brood centre, at which each defence behaviour ended, was recorded. All observed defence behaviours were located within the marked 150 cm radius and fry did not shift positions during these observations.

First, we ascertained that intrusion pressure on territories did not differ between treatments by using paired Wilcoxon signed-rank tests to compare the number of defence behaviours (as proxy for number of intrusions, see experiment 2) before and after male removal. Then, we tested for differences in defence distance between females and males before male removal. To do this, we fitted an LMM (R package lme4; Bates et al., 2015) with defence distance (for each defence act) as the response variable. We included sex of the defender as a predictor variable along with 'body size' as covariate. 'Territory ID' nested within 'date of first observation' was included as a random intercept to account for nonindependence of data. Next, we compared the females' defence distances before and after male removal. We fitted an LMM with defence distance of the female as the response variable. We included 'pair status' as a predictor variable indicating whether the distances were measured before or after male removal. We also included 'female body size' as covariate and 'territory ID' nested within 'date of first observation' as random intercept.

Ethical Note

This study was carried out with the approval of the ethics committee of the University of Graz (permit number 39/50/63 ex 2018/19). Fieldwork was carried out with the permission of the Fisheries Department of Zambia and under study permits issued by the government of Zambia (SP 007214, SP 007215, SP 007216, SP 007217, SP 008735). The study species is listed as 'Least Concern' under the name of *Neolamprologus moorii* in the IUCN Red List of Threatened Species 2006. All fin clips were used as DNA samples for genetic parentage analysis in this and other studies. All fish handling and fin clipping were performed by trained personnel, and previous research indicates that adult *V. moorii* resume normal territorial behaviour within minutes of such handling (Zimmermann et al., 2019).

RESULTS

Experiment 1: Brood Survival after Parent Removal

To monitor fry loss in territories with uniparental care, we counted fry before the removal of one parent and then again 1 - 4 days afterwards. We note that the sampling of fry for parentage analysis, which had been conducted in a subset of nests prior to female removal, did not lead to significantly more fry losses by uniparental males (GLMM: estimate ± SE = .90 ± 1.29, $t_{23} = 1.47$, $P = 0.17$). Twenty of the divorced males (out of 33), but none of the divorced females (out of 19), lost their entire brood. Males also lost significantly higher proportions of their broods than females (males: median = 100% fry loss, range 0–100%, $N = 33$; females: median = 10% fry loss, range 0–47%, $N = 19$; GLMM: estimate ± SE = -3.09 ± 0.68, $t_{38} = -4.55$, $P = 0.0001$; Fig. 2a). Within our sample, males were on average 4.5% smaller than their paired females (mean ± SD: males = 8.4 ± 0.6 cm, females = 8.8 ± 0.5 cm; two sample t test: $t_{37.82} = -2.51$, $P = 0.02$). The GLMM estimating the sex difference also included the parent's body size, brood size and the duration of
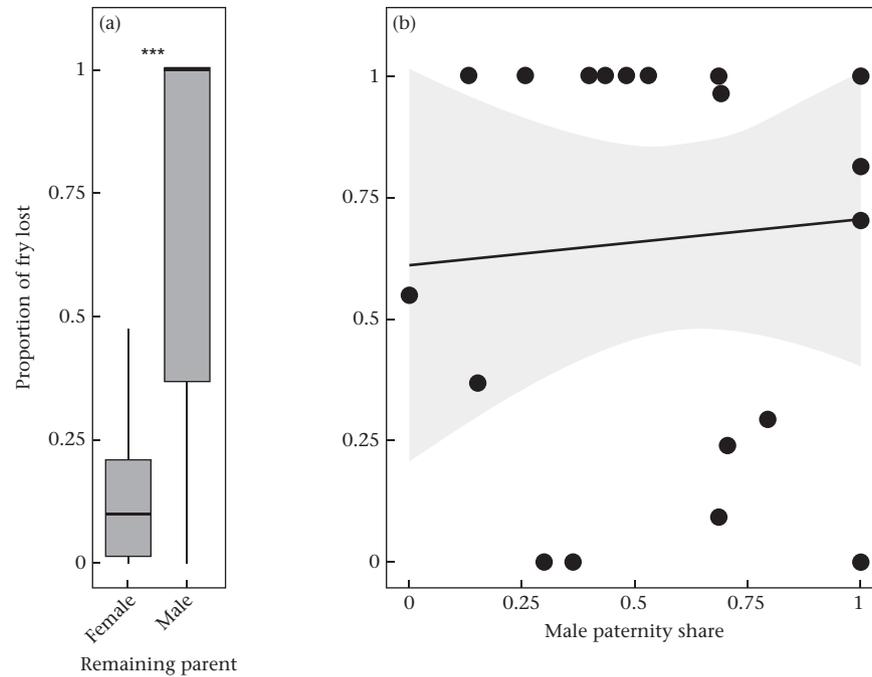
Figure 2. (a) Fry loss suffered by experimentally divorced females and males. Box plots show medians (thick vertical lines), first and third quartiles (boxes) and the range of data within 1.5 interquartile distances above and below the interquartile (whiskers). (b) The relationship (ordinary linear regression fitted with 95% confidence interval) between fry loss and the social mate's paternity share after removal of the female. ***$P < 0.001$.

uniparental brood care (i.e. the interval between mate removal and fry counting), none of which had an effect on fry loss (GLMM: parent size: estimate $\pm$ SE = 0.30 $\pm$ 0.30, t_{38} = 0.99, P = 0.33; brood size: estimate $\pm$ SE = -0.01$\pm$ 0.01, t_{38} = -0.67, P = 0.51; duration: estimate $\pm$ SE = 0.02 $\pm$ 0.02, t_4 = 1.12, P = 0.32).

Among fish that were monitored for 3 or 4 days after removing their mate, seven of the 28 males and none of the 14 females disappeared from their territories (Fisher's exact test: P = 0.08). For fish that retained their territories over this same period, the difference in fry loss between males and females was significantly higher in males than in females (males: median + interquartile range, IQR = 81.5 + 38.4% fry loss, range 0–100%, N = 21; females: median + IQR = 18.5 + 13% fry loss, range 0–47%, N = 14; GLMM: estimate $\pm$ SE = -2.29 $\pm$ 0.65, t_5 = -3.52, P = 0.02), with no significant effects of the covariates parent size, brood size and duration of uniparental brood care (GLMM: parent size: estimate $\pm$ SE = 0.33 $\pm$ 0.32, t_{25} = 1.05, P = 0.31; brood size: estimate $\pm$SE = -0.00 $\pm$ 0.01, t_{25} = -0.10, P = 0.92; duration: estimate $\pm$ SE = 0.10 $\pm$ 0.04, t_5 = 2.36, P = 0.07). Similarly, the proportion of single parents that retained their territories but lost their broods completely was higher in males than in females (males: 10 of 21; females: 0 of 14; Fisher's exact test: P = 0.002). None of the divorced individuals paired with new partners within the experimental duration.

We also tested for a correlation between fry loss and paternity in 21 nests (from which broods had been subsampled for paternity testing). Paternity shares in these broods ranged from 0 to 100% (median + IQR = 68.7 + 63.6%) and fry losses also ranged from 0 to 100% (median + IQR = 96.7 + 70.6%). However, we detected no significant relationship between paternity and fry losses (GLMM: estimate $\pm$ SE = -0.19 $\pm$ 2.04, t_{12} = -0.09, P = 0.93; Fig. 2b), and no significant effects of the covariates parent size, brood size and duration of uniparental brood care (GLMM: parent size: estimate $\pm$ SE = 0.62 $\pm$ 0.66, t_{12} = 0.93, P = 0.37; brood size: estimate $\pm$ SE =0.02 $\pm$ 0.04, t_{12} = 0.60, P = 0.56; duration: estimate $\pm$ SE = -0.35 $\pm$ 4.07, t_2 = -0.09, P = 0.94).

Experiment 2: Division of Defence Changes after Brood Removal

Here, we counted defence behaviours performed by both males and females before and after the removal (N = 14 territories) or sham removal (N = 14 territories) of the brood. Total defence rates (numbers of defence behaviours summed across the male and female partner, rendered per min observation time) reflect the intrusion pressure on the territory. The treatment groups did not differ significantly in total defence rates before the brood was removed (Wilcoxon signed-rank test: V = 61, P = 0.29). Parents in the brood removal group performed a median of 2.6 (IQR = 1.4, range 1.1–4.0) defence behaviours/min before brood removal and this dropped significantly to 1.5 (IQR = 0.6, range 0.6–2.5) defence behaviours/min after brood removal (paired Wilcoxon signed-rank test: V = 95, P = 0.01). In contrast, rates of defence behaviours in the control group did not change after sham removal of the brood (before sham removal: median + IQR = 1.7 + 0.8, range 0.5–3.5; after sham removal: median + IQR = 1.7 + 0.9, range 0.8–4.3; paired Wilcoxon signed-rank test: V = 38.5, P = 0.65).

Next, we investigated female share in defence (i.e. the proportion of total defences that was performed by the female), and found a significant interaction between treatment group (removal versus sham removal) and time of observation (before versus after manipulation; GLMM: interaction, estimate $\pm$ SE = 0.43 $\pm$ 0.22, z = 1.98, P = 0.05). To begin with, female share in defence did not differ between the brood removal and the control groups prior to the manipulation (estimate $\pm$ SE = -0.11 $\pm$ 0.17, z = -0.66, P = 0.51; Fig. 3). However, females in the brood removal group significantly decreased their share in defence, from 55 $\pm$ 14% (mean $\pm$ SD) before brood removal to 39 $\pm$ 13% after brood removal (estimate $\pm$ SE = -0.43 $\pm$ 0.17, z = -2.59, P = 0.01; Fig. 3), whereas females in the control group did not significantly change their share in defence between time points, performing 54 $\pm$ 13% (mean $\pm$ SD) of the total defence behaviours before sham removal and then 50 $\pm$ 13% after sham removal (estimate $\pm$ SE = 0.00 $\pm$ 0.15, z = -0.00, P = 1.00; Fig. 3). Female share in defence was positively correlated with the

mean body size of the paired individuals (estimate $\pm$ SE = 2.16 $\pm$ 1.05, z = 2.05, P = 0.04).

Experiment 3: Male Behaviour after Female Removal

Here, we examined how female removal affected male space use on the territory and the males' position relative to the brood. Ten of the 14 divorced males remained on their territories, and six of these also retained a portion of their broods over the experimental period. While paired, the average ($\pm$ SD) distance of males from their broods was 40.0 $\pm$ 31.0 cm (N = 14 males). After female removal, these distances were significantly reduced to a mean of 11.8 $\pm$ 15.6 cm in the six males that still possessed a brood (LMM: estimate $\pm$ SE = -21.64 $\pm$ 5.02, $t_{19.72}$ = -4.31, P = 0.0004).

Using the grid of marker stones positioned on the territories, we calculated the areas of utilized space for each male that remained on their territories after female removal (N = 10). Both the total areas (99.9% UD, mean $\pm$ SD = 1.12 $\pm$ 0.68 m^2) and the core areas (50% UD, 0.09 $\pm$ 0.07 m^2) used by the males decreased significantly after female removal (LMM: 99.9% UD: 0.71 $\pm$ 0.3 m^2, estimate $\pm$ SE = -0.4 $\pm$ 0.15, t_9 = -2.73, P = 0.02; 50% UD: 0.04 m^2 $\pm$ 0.02 m^2, estimate $\pm$ SE = -0.05 $\pm$ 0.02, t_9 = -2.51, P = 0.03). Because only 10 males remained on their territory, we did not distinguish between males that lost (four males) and males that retained their brood (six males) in this analysis. Fig. 4 shows that both types of males reduced their space use after female removal.
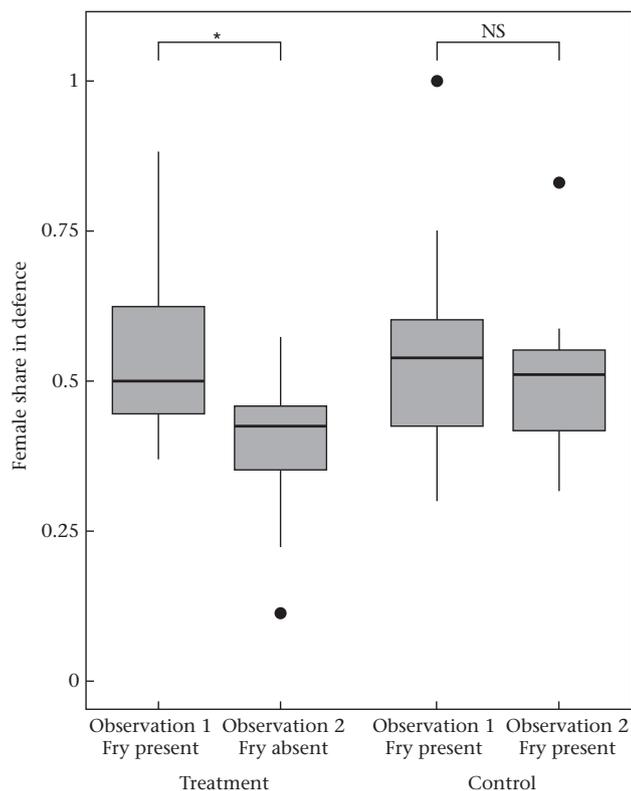

Figure 3. Female share in defence before and after fry removal (treatment) and sham removal (control). Female share represents the proportion of all defence actions that was carried out by the female during 15 min of territory observation. Observation 1: observation before (sham) removal of fry; observation 2: observation after (sham) removal of fry. Box plots show medians (thick vertical lines), first and third quartiles (boxes), the range of data within 1.5 interquartile distances above and below the interquartile (whiskers) and individual outliers. *P < 0.05.

Experiment 4: Reduction in Defence Distances after Male Removal

Here, we asked whether male defence contributes to the safety provided to the fry and tested for changes in the distance from the brood at which intruders were attacked and repelled after the male was removed from the territory. Pairs of brood-tending *V. moorii* performed 7–33 defence actions per 10 min against approaching conspecific and heterospecific fish (median + IQR = 18.5 + 8). The intrusion pressure did not differ before and after male removal (Wilcoxon signed-rank tests: V = 388.5, P = 0.78). The mean ($\pm$ SD) defence distance of brood-tending *V. moorii* pairs was 46 $\pm$ 13 cm (range 30–130 cm, N = 14 nests). Within pairs, females defended at shorter distances than males (mean $\pm$ SD; females = 43.17 $\pm$ 13.25 cm, males = 48.36 $\pm$ 12.62 cm; LMM: estimate $\pm$ SE = -5.72 $\pm$ 0.10, $t_{582.24}$ = -5.74, P < 0.0001; Fig. 5), and there was a trend towards a positive correlation between defence distances and body size across both sexes (estimate $\pm$ SE = 2.71 $\pm$ 1.51, $t_{99.31}$ = 1.79, P = 0.08). After removal of the male, females defended their broods at a distance of 36 $\pm$ 12 cm (range 30–110 cm). The average defence distance of single females was 20% shorter than when they had still been paired (LMM: estimate $\pm$ SE = -7.11 $\pm$ 1.27, $t_{72.51}$ = -5.58, P = 3.9 $\times$ 10^{-7}; Fig. 5). Independent of pair status, female defence distance was positively correlated with body size (estimate $\pm$ SE = 4.59 $\pm$ 1.39, $t_{73.47}$ = 3.31, P = 0.001).

DISCUSSION

When parents attack intruders encroaching on a nesting site or a territory, their behaviour is often ascribed to offspring protection, and thus considered a form of parental care. However, when territories or nests are reused across brood cycles or serve multiple purposes (e.g. foraging, mate attraction, etc.), the factors that drive defence behaviour can be difficult to disentangle. While defence often benefits broods, other drivers such as territory retention can also provide powerful incentives (Smiseth et al., 2012; Whiteman & Côté, 2004). Furthermore, in species with biparental care, broods may be more valuable to one parent than to the other, and even if both parents participate in defence, the drivers underlying their behaviour may not be the same. We developed the following predictions for the case that male and female defence behaviours are equally driven by brood survival: males and females should be equally successful in uniparental brood defence (rejected by experiment 1) and division of defence labour between males and females of a pair should be independent of the presence of the brood (rejected by experiment 2). Taken together, the results of these experiments and of Zimmermann et al.'s (2019) study suggest that in *V. moorii*, female defence behaviour is driven more by offspring protection relative to males, while male defence during biparental brood care may be primarily aimed at territory retention. Prompted by the males' poor performance in brood defence, we investigated whether males respond behaviourally to the removal of their mates (reduction of space use demonstrated by experiment 3), and whether the males' presence on the territory confers benefits to the brood (effect on brood predation risk suggested by experiment 4).

In experiment 1, the divorced males suffered considerably higher rates of brood loss compared to divorced females. More than half of the broods under uniparental male care (60%) disappeared completely within 1–4 days, whereas divorced females suffered only minor, if any, fry losses. There was a trend for divorced males to lose or abandon their territories more often than divorced females, but this did not explain the excessive brood losses suffered by divorced males. Even among those fish that managed to retain their territories, males lost significantly more fry than females. Given that male and female *V. moorii* are similar in body size and

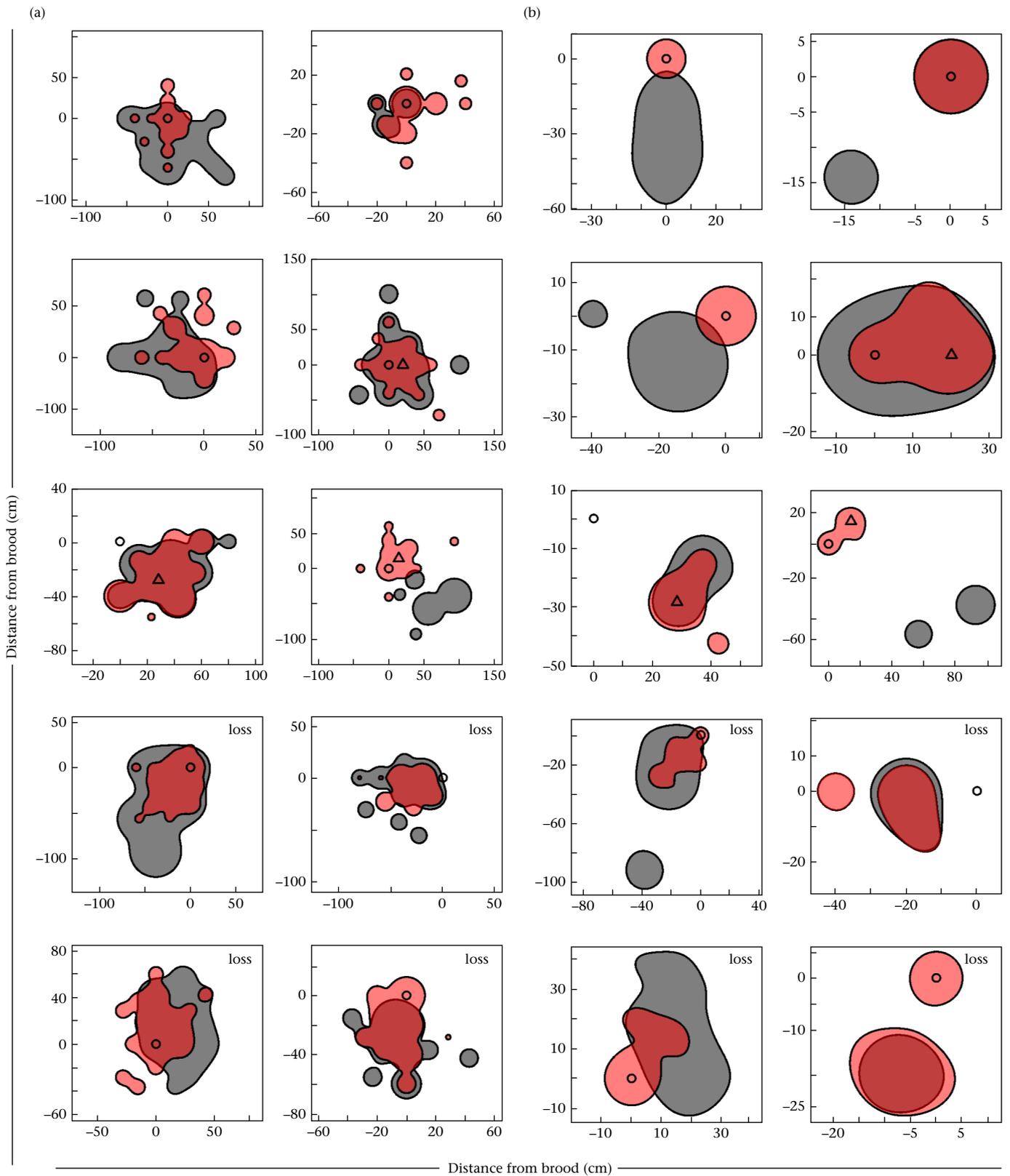**Figure 4.** Changes in male (a) whole areas (99.9% utilization distribution, UD) and (b) core areas (50% UD) before (grey) and after (red) female removal. Circles represent the centre of the space occupied by the brood. Some broods changed their position after female removal. When this happened, their new positions are indicated by triangles. 'Loss' indicates territories where fry disappeared after female removal.
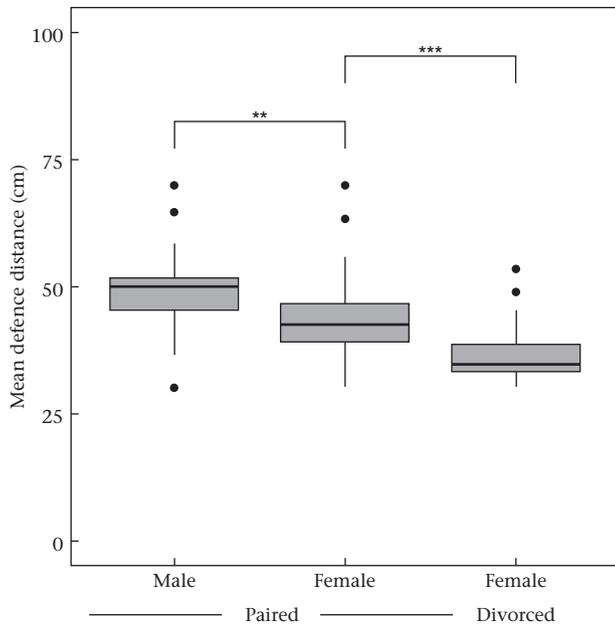
Figure 5. Defence distances of males and females while paired (before male removal) and of divorced females (after male removal). The distances from the brood at which intruders were attacked were averaged across observation sessions. Box plots show medians (thick vertical lines), first and third quartiles (boxes), the range of data within 1.5 interquartile distances above and below the interquartile (whiskers) and individual outliers. **$P < 0.01$; ***$P < 0.001$; note that the contrast between paired males and divorced females was not tested for significance.

morphology and attack the same range of intruder species (see above), we suggest that differences in offspring survival probably reflect different levels of commitment to the brood rather than different levels of defence capabilities. While we acknowledge that disturbances caused by the subsampling of fry (for paternity testing) and temporal variation among territories in when treatments were applied (Fig. 1) may have influenced the parental behaviour of males differently than females, comparisons between subsampled and non-subsampled territories indicated that rates of fry loss were not associated with these disturbances. Therefore, although our observation regime differed between males and females, it seems unlikely that the large difference between male and female fry losses could be attributable to this variation. *Variabilichromis moorii* parents must contend with intrusions from a wide array of heterospecifics, which vary in their threat level towards offspring. When both parents are present on the territory, males have previously been shown to contribute less than females to defence against potential offspring predators but contribute equally to defence against potential territory competitors (Zimmermann et al., 2019). Thus, our removal of females also represented a removal of the primary defenders against predatory heterospecifics. Our data suggest that divorced males provided less (or less effective) brood defence than divorced females. However, we cannot exclude an alternative possibility that fry losses were partly due to filial cannibalism by the remaining parent. Either way, the difference in brood losses between the sexes suggests that males were less committed to invest in the survival of their brood than females were. Furthermore, we detected no correlation between paternity and brood losses under male-only care. This is congruent with a previous study, which showed that male contributions to biparental defence are similarly not correlated with paternity (Zimmermann et al., 2019). Altogether, low average

paternity across evolutionary time along with cuckoldry opportunities throughout a male's lifetime may have dampened male parental investment to the point where males of this species do not respond to paternity cues and fail to guarantee brood survival when left as single parents.

Parent removal experiments have been conducted in a wide range of taxa (e.g. Smiseth, Dawson, Varley, & Moore, 2005; Suzuki & Nagano, 2009). In birds, such experiments have generally demonstrated that the remaining parent, regardless of sex, will increase its incubation and/or provisioning and consequently complete brood loss is not common (Harrison, Barta, Cuthill, & Székely, 2009; Leniowski & Węgrzyn, 2018). In the burying beetle *N. vespilloides*, males increased their time spent on parental duties after female removal and uniparental care had no negative effect on offspring survival (Smiseth et al., 2005). In fishes, single females of otherwise biparental species have been observed to successfully defend and care for their broods under field conditions (*Amphilophus sagittae* and *Amphilophus zaliosus*, Lehtonen et al., 2011; *A. nigrofasciata*, Wisenden, 1994; *Hypsophrys nematopus*, Sowersby et al., 2018), although sometimes experiencing fry losses (*A. nigrofasciata*, Wisenden & Keenleyside, 1992). In contrast, reports of single males doing so are rare. In *A. nigrofasciata*, males increased their defence behaviour against territory intruders after experimental removal of their female partners (van Breukelen & Itzkowitz, 2011), but the consequences for brood survival were not reported. Overall, the high rate of brood failure for males observed in our study may represent a rather extreme outcome for enforced uniparental care in an otherwise putatively biparental species.

Numerous studies have shown that animals adjust their fight and defence behaviour according to the value of the contested resources, such as territories, food or offspring (Arnott & Elwood, 2007; Dearborn, 1998; Johnsson, Carlsson, & Sundstrom, 2000; McCallum, Gulas, & Balshine, 2017; Rytkönen, 2002). Likewise, the extent to which breeders contribute to brood care in biparental systems can reflect how valuable the brood is for each of the two parents (Fromhage & Jennions, 2016; Jennions & Polakow, 2001; Suter, Bielanska, Rothlin-Spillmann, Strambini, & Meyer, 2009). In *V. moorii*, brood removal was expected to impact females to a greater degree than males. Indeed, the relative contributions of females to territory defence decreased significantly after brood removal in experiment 2. This implies that the loss of value associated with brood removal (i.e. from the combined value of territory plus brood to the value of the territory alone) was greater for females than for males. The sample size in the brood removal experiment was limited to only 14 pairs, but the conclusion drawn from these data is consistent with the results of a previous experiment, in which paired males of *V. moorii* contributed less to defence against brood predators than against territory competitors (Zimmermann et al., 2019).

Sex-specific brood care behaviour has been observed in several biparental fish species (Itzkowitz et al., 2001; McKaye, Mughogho, & Stauffer, 1994; McKaye & Murry, 2008; Rogers, 1988), including in cichlids, with females staying closer to the brood than their male partners (Keenleyside, 1991; Nakano & Nagoshi, 1990). In experiment 3, males reduced their average distance to the brood after female removal. The divorced males also reduced their areas of utilized space, and this reduction was observed regardless of whether the offspring were still present or had disappeared (Fig. 4). Staying closer to the brood is consistent with what we expect to observe if males adjust their behaviour to the demands of brood care. However, the space use reductions, which also occurred when the brood was lost, suggested that divorced males may simply not

be able to defend as large a territory as they could prior to female removal and future work will be required to assess this possibility.

In experiment 4, male removals resulted in intruders encroaching deeper into the territories, and thus closer to the broods, before being repelled by the remaining female parent. Parents of *Lamprologus toae*, another biparental cichlid species in Lake Tanganyika, attack potential fry predators at much greater distances than intruders of nonpredatory species, and the most proficient predators (*Lepidiolamprologus* spp., which are also frequent intruders on *V. moorii* nests, Zimmermann et al., 2019) were attacked from even greater distances than other predatory species (Nakano & Nagoshi, 1990). This adjustment to intruder type suggests that defence distances represent a balance between distance-dependent costs (e.g. energetic expenditure, temporarily constrained attention) and benefits (e.g. preventing predation), with frequent close approaches probably increasing brood predation risk. As in our study, a pair's average defence distance has previously been used as a proxy for parents' reaction times towards brood threats and, hence, has served as a measure of brood care quality in the context of biparental versus uniparental comparisons in Neotropical cichlids (Lehtonen et al., 2011; Sowersby et al., 2018). Since intrusion pressure on *V. moorii* territories did not differ before and after male removal, the decrease in average defence distance suggests that divorced females provide poorer quality defence in the absence of their male partner (as suggested by Lehtonen et al., 2011; Sowersby et al., 2018).

In this paper, we have presented a series of field experiments aimed at elucidating sex differences in the drivers behind defence behaviours in a cichlid with putatively biparental brood care. The challenges of conducting behavioural experiments in the wild can sometimes lead to suboptimal sample sizes and study designs, but despite these hurdles and when viewed in the light of previous findings (e.g. Bose et al., 2018; Zimmermann et al., 2019), our results collectively suggest that male defence behaviour is less strongly driven by brood protection than female defence behaviour. The males' focus on defence against territory competitors (Zimmermann et al., 2019) and their larger shares in territory defence when broods are absent suggest that males are more committed to territory protection than to brood care. Although not all males retained or remained on their territory after being divorced, these insights help to reconcile the considerable defence efforts made by males in this species with the high rates of paternity loss that they suffer from cuckolders.

Importantly, offspring probably benefit from males' defence efforts regardless of the primary drivers of male defence behaviour. Our results suggest that, relative to female defence, male defence in *V. moorii* aligns less well with standard definitions of 'parental care' (e.g. Smiseth et al., 2012), even though males and females perform similar behaviours. We propose that sex differences in the drivers underlying defence behaviours may extend to other biparental breeders, particularly among fishes in which high rates of cuckoldry may reduce male investment in offspring, but defence still pays due to the benefits of territoriality.

Supplementary Material

Supplementary material associated with this article can be found online at https://doi.org/10.1016/j.anbehav.2021.03.012.

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Appendix

Table A1
Variabilichromis moorii territories used in the experiments

ID	Year	Month	Fry size (mm)	Brood size at start of treatment	Depth (m)	Male size (cm)	Female size (cm)	Paternity	Experiments
nest31	2015	Oct	NA	35	1.3	8.1	NA	NA	1 (MR)
nest35	2015	Oct	NA	37	1.7	8.1	8	NA	1 (MR)
nest39	2015	Oct	NA	29	1.9	8.2	8.3	NA	1 (MR)
nest 27	2015	Oct	NA	45	2	8.3	NA	NA	1 (MR)
nest 16	2015	Oct	NA	36	3.6	8.8	9.2	NA	1 (MR)
nestM1	2015	Oct	NA	20	5.5	8.7	8.6	NA	1(FR)
nestM5	2015	Oct	NA	24	5.9	8.8	8.3	NA	1(FR)
nestM7	2015	Oct	NA	11	4.7	8.4	8.4	NA	1(FR)
nestM2	2015	Oct	NA	67	8.1	9.5	9.2	NA	1(FR)
nestM4	2015	Oct	NA	14	7.4	8.4	8.5	NA	1(FR)
nestB7	2017	Oct	6	85	3.4	8.8	9	0.687	1(FR, PA), 3
nest7	2017	Oct	7	68	5.1	8	8.5	0.692	1(FR, PA)
nest6	2017	Oct	7	29	5.2	8.6	9	0.708	1(FR, PA), 3
nest2	2017	Oct	NA	16	2.2	8.3	7.7	NA	1(FR), 3
nest 10	2017	Oct	NA	46	4.2	8.4	9.4	NA	1(FR), 3
nest38	2017	Oct	NA	11	4.9	8.3	8.9	NA	1(FR), 3
nest37	2017	Oct	NA	19	3	8	7.9	NA	1(FR), 3
nest36	2017	Oct	15	51	3.1	8	7.9	0.794	1(FR, PA), 3
nest34	2017	Oct	NA	19	3.7	8.8	7.5	NA	1(FR)
nest4	2017	Oct	22	36	3.6	8.8	8.9	0.300	1(FR, PA), 3
nest39	2017	Oct	7	56	5.5	8.9	9	0.531	1(FR, PA)
nest 29	2017	Oct	19	51	4.4	9	9.5	1.000	1(FR, PA), 3
nest32	2017	Oct	NA	18	2.5	7.6	7.6	NA	1(FR), 3
nest 27	2017	Oct	NA	30	2.1	7.9	8	NA	1(FR)
nest 22	2017	Oct	NA	90	5.3	8.5	8.7	NA	1 (MR), 4
nest 13	2017	Oct	NA	43	3.4	7.6	8.4	NA	1 (MR), 4
nest 19	2017	Oct	19	119	4.5	8.6	9.2	NA	1 (MR), 4
nest1	2017	Oct	20	83	1.5	8.7	9.2	NA	1 (MR), 4
nest 16	2017	Oct	NA	10	2.6	7.8	8	NA	1 (MR), 4
nest9	2017	Oct	18	12	3.6	8.4	8.5	NA	1 (MR), 4
nest 14	2017	Oct	NA	15	4.4	8.7	8.6	NA	1 (MR), 4
nest5	2017	Oct	21	64	4.7	8.8	9	NA	1 (MR), 4
nest8	2017	Oct	14	47	4.5	9.6	9.9	NA	1 (MR), 4
nest 28	2017	Oct	12	90	4.6	8	9.2	NA	1 (MR), 4
nest30	2017	Oct	NA	31	4.4	9.1	9.2	NA	1 (MR), 4
nestE2	2017	Oct	18	53	5	9.2	9.3	NA	1 (MR), 4
nest 23	2017	Oct	NA	29	1.6	8.9	8.8	NA	1 (MR), 4
nest31	2017	Oct	NA	19	2.7	8.8	8.6	NA	1 (MR), 4
nestF2	2018	Apr	6	13	3	8.7	8.4	0.133	1(FR, PA)
nestF3	2018	Apr	15	7	2.9	7.6	8.8	0.257	1(FR, PA)
nestF5	2018	Apr	6	15	3.2	8.1	8.6	0.483	1(FR, PA)
nestF6	2018	Apr	12	11	3.3	7.4	8.3	0.688	1(FR, PA)
nestF7	2018	Apr	12	13	5.5	7.7	8	0.364	1(FR, PA)
nestF8	2018	Apr	10	20	2.1	7.7	8.2	0.435	1(FR, PA)
nestF10	2018	Apr	18	38	6.7	9.2	10.9	0.153	1(FR, PA)
nestF12	2018	Apr	16	20	7.1	9.1	8.3	0.000	1(FR, PA)
nestFR1	2018	Sept	31	27	2.5	7.5	8	1.000	1(FR, PA)
nestFR2	2018	Sept	22	27	4.2	8.2	8.4	1.000	1(FR, PA)
nestFR3	2018	Sept	30	35	5.1	8.6	8.9	1.000	1(FR, PA)
nestFR4	2018	Sept	19	27	5.6	9.6	8.7	0.400	1(FR, PA)
nestFR6	2018	Sept	24	66	4.4	8.7	9.5	1.000	1(FR, PA)
nestFR7	2018	Sept	23	37	5.3	9.6	8.6	1.000	1(FR, PA)
nestI1	2018	Apr	NA	NA	6.3	8.7	10.1	NA	2(C)
nestI4	2018	Apr	NA	NA	5.9	7.2	7.5	NA	2(C)
nestI5	2018	Apr	NA	NA	5.4	7.0	7.4	NA	2(C)
nest 28	2018	Apr	NA	NA	6.5	8.8	8.3	NA	2(C)
nestI2	2018	Apr	NA	NA	5	7.7	8.2	NA	2(C)
nestI 10	2018	Apr	NA	NA	5.8	7.9	9.4	NA	2(C)
nestI 13	2018	Apr	NA	NA	4.3	7.6	7.6	NA	2(C)
nestI 15	2018	Apr	NA	NA	6.7	8.5	8.5	NA	2(C)
nestI 18	2018	Apr	NA	NA	5	7.5	7.6	NA	2(C)
nestI 19	2018	Apr	NA	NA	6.9	8.2	9.6	NA	2(C)
nestI 24	2018	Apr	NA	NA	6.4	7.5	9.5	NA	2(C)
nestI 26	2018	Apr	NA	NA	5.9	7.6	7.6	NA	2(C)
nestI 27	2018	Apr	NA	NA	6.5	8.4	8.3	NA	2(C)
nestI 29	2018	Apr	NA	NA	6.4	8.6	9.5	NA	2(C)
nestA2	2018	Apr	13	21	4.4	7.2	8.6	NA	2
nestI3	2018	Apr	11	4	6.2	8.1	9.1	NA	2
nestI6	2018	Apr	10	5	4.9	7.6	8.3	NA	2

Table A1 (*continued*)

ID	Year	Month	Fry size (mm)	Brood size at start of treatment	Depth (m)	Male size (cm)	Female size (cm)	Paternity	Experiments
nestl7	2018	Apr	14	58	4.4	8.0	8.5	NA	2
nestl8	2018	Apr	10	48	5.3	8.0	8.5	NA	2
nest30	2018	Apr	10	9	6.1	8.0	8.1	NA	2
nestl 14	2018	Apr	9	64	6.6	8.3	9.4	NA	2
nestl 16	2018	Apr	14	9	4.6	7.8	7.6	NA	2
nestl 17	2018	Apr	9	25	4.9	8.7	8.3	NA	2
nestl 20	2018	Apr	13	31	4.4	7.1	8.1	NA	2
nestl 21	2018	Apr	15	53	6.5	8.0	8.3	NA	2
nestl 22	2018	Apr	11	8	6.6	8.5	7.9	NA	2
nestl 28	2018	Apr	12	63	6.3	8.0	8.3	NA	2
nestC	2018	Apr	5	97	6.6	7.7	7.8	NA	2

Paternity denotes the proportion of fry sired by the nest-tending male. Experiments are labelled 1−4 as in the Methods. Within experiments, MR: male removal group; FR: female removal group; C: control group; PA: sampled for genetic paternity analysis.