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Reproductive suppression in female cooperatively breeding cichlids

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Suppression by dominants of female subordinate reproduction has been found in many vertebrate social groups, but has rarely been shown experimentally. Here experimental evidence is provided for reproductive suppression in the group-living Lake Tanganyika cichlid *Neolamprologus pulcher*. Within groups of three unrelated females, suppression was due to medium- and small-sized females laying less frequently compared with large females, and compared with medium females in control pairs. Clutch size and average egg mass of all females depended on body size, but not on rank. In a second step, a large female was removed from the group and a very small female was added to keep the group size constant. The medium females immediately seized the dominant breeding position in the group and started to reproduce as frequently as control pairs, whereas clutch size and egg mass did not change. These results show that female subordinate cichlids are reproductively capable, but apparently suppressed with respect to egg laying. Nevertheless, some reproduction is tolerated, possibly to ensure continued alloparental care by subordinate females.

Keywords: reproductive competition; cooperative breeding; dominance; body size; Cichlidae

1. INTRODUCTION

Reproductive suppression of subordinate reproduction has been reported in cooperatively breeding vertebrates and may come about by active dominant suppression (e.g. Hoogland 1985) or inbreeding avoidance (e.g. Clarke *et al.* 2001), but experimental studies are limited. Dominant reproductive suppression has to be shown while holding other effects (e.g. female body size) constant, and individuals should increase reproduction after a rise in rank from subordinate to dominant.

Reproductive suppression in female cooperatively breeding fish has not been studied, but subordinate females do reproduce (Heg & Hamilton 2008). Here I present an experimental study of female reproductive suppression in unrelated groups of the cichlid *Neolamprologus pulcher*, by comparing reproduction of similar-sized females of different ranks, and by manipulating rank itself. I compare reproduction of these cichlids depending on their size-based dominance rank inside groups of three females of different sizes (rank 1 to rank 3, largest to smallest female). To control for body size effects, I then compare subordinate reproduction (rank 2) with same-sized dominant females living in pairs (rank 1). To test for rank effects, I compare subordinate reproduction before (when they are ranked 2) versus after (when they have acquired rank 1) the dominant female has been removed from its group. If the latter two tests show suppressed subordinate reproduction, this effect must be due to dominant suppression. I then show that, although subordinates care mainly for their own broods, their alloparental care may reduce the parental care load of higher ranking females, suggesting some subordinate reproduction may be conceded to ensure continued subordinate helpfulness.

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2. MATERIAL AND METHODS

I created artificial groups of four unrelated individuals. I created 32 groups, each with a male and three females (large, medium and small); and eight control pairs, each with a male and a medium female (figure 1*a*). I measured the sizes of the fish at the start of each sequence (body mass in mg and standard length SL in mm, see electronic supplementary material), sexed them by examining the genital papilla and marked all the fish individually. At the end of sequence 1 (1 month), the largest females in each group were all replaced with another large breeding female (start of sequence 2, duration also 1 month). The female dominance ranks during sequences 1 and 2 were large rank 1, medium rank 2 and small rank 3. After sequence 2, the large female in each group was removed and replaced with a very small female (sequence 3). Now the female dominance ranks within groups were medium rank 1, small rank 2 and very small rank 3. Control pairs contained a medium-sized breeding female throughout all three sequences and were not replaced (these medium females had rank 1).

Groups and pairs were checked daily for courtship, spawning and brood care activity. After spawning was completed, maternal brood care and alloparental brood care were determined for all the group members simultaneously during a 15-min observation period (frequency of cleaning and fanning eggs combined). In the evening, the clutches were removed, eggs counted and average egg mass was determined (see electronic supplementary material).

Results were analysed using generalized estimating equations (GEE) in SPSS 15, which allows for repeated measurements of the same subjects (individual identifier entered as subject, see electronic supplementary material).

3. RESULTS

Subordinate female cichlids appeared to be reproductively suppressed, i.e. they produced fewer and smaller clutches in the presence of a higher ranking female (figure 1). Three lines of evidence showed that the suppression in the number of clutches appeared to be due to the presence of a higher ranking female, whereas the suppression in clutch size was caused by rank-related differences in body size. First, when body size and body condition effects were statistically controlled for, rank still had a significant effect on the number of clutches (figure 1, table 1, both the medium and small versus large females: $p<0.001$; medium versus small females: $p=0.71$). By contrast, clutch size and egg mass were solely affected by female body size, but not by their rank (table 1, all comparisons of rank $p>0.10$). The medium females tended to produce the largest clutches for their size, whereas the small females tended to produce the heaviest eggs for their size, in contrast to what would be expected from reproductive suppression by the large females (table 1).

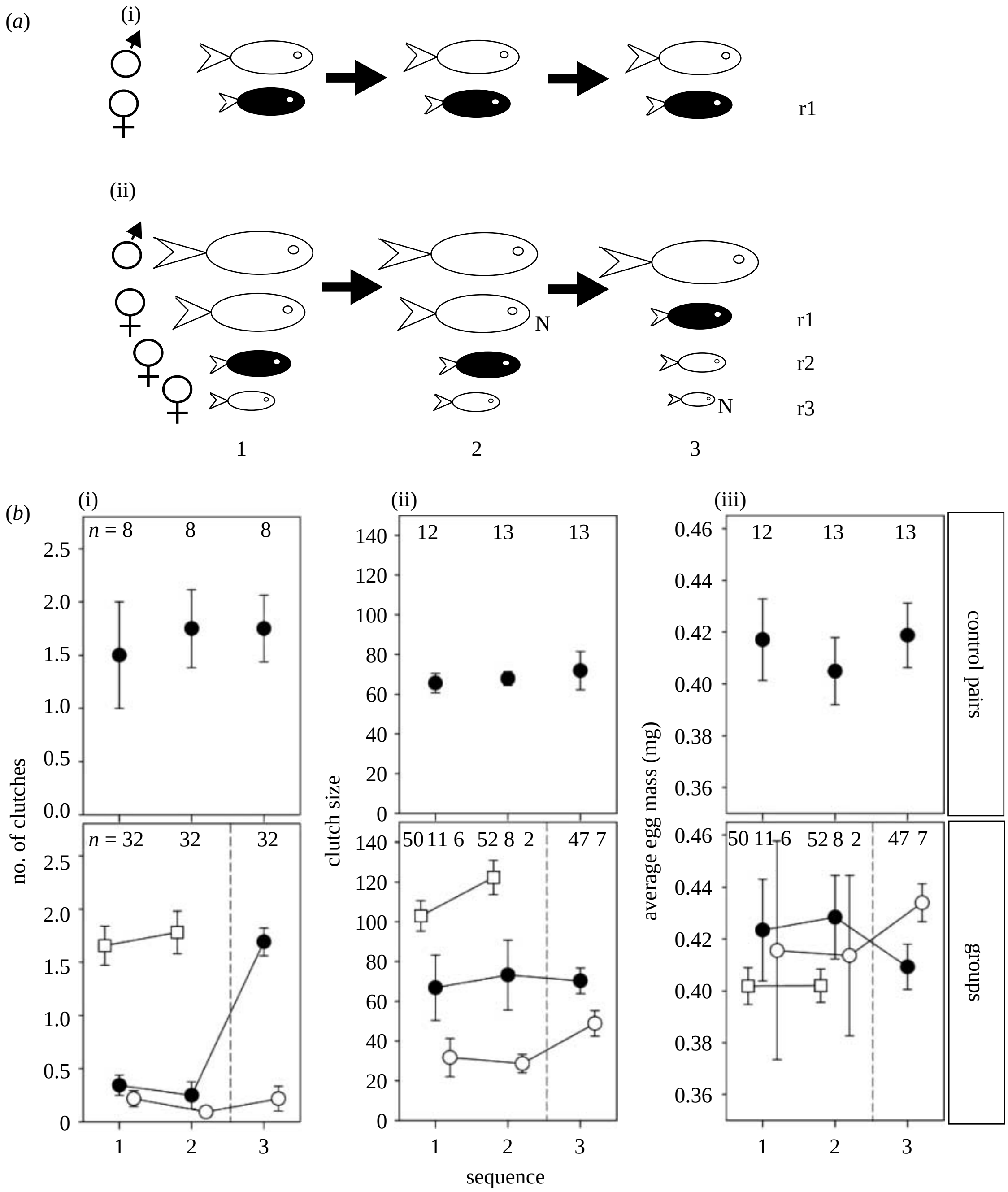

Figure 1. (*a*) Design of the experiment. In control pairs (i), medium females (black) were breeding with a male throughout all three sequences. In groups (ii), medium females (black) and small females were with a large female and male in sequences 1 (for 30 days) and 2 (for 30 days). Note that large females were exchanged in between (indicated with 'N' for new). After sequence 2, all large females were permanently removed and a very small helper female was added (indicated with 'N') and sequence 3 lasted another 30 days. Note that this removal resulted in changes of female rank (r1, r2, r3). (*b*) Reproductive suppression in female *N. pulcher*. Depicted are means ± s.e. of the number of clutches, clutch size and average egg mass. Note that medium females (filled circles; r2–r1) and small females (open circles; r3–r2) experienced a rank change from sequence 2 to 3 (indicated with vertical dashed line). Very small females (rank 3) did not produce any clutches during sequence 3 and are omitted for clarity. Sample sizes are indicated inside the panels (*b*(i): n = number of individuals; *b*(ii) and *b*(ii): n = number of clutches). Squares, large females.

Second, the medium females living in groups ('rank 2') produced significantly fewer clutches than the medium females breeding in pairs ('rank 1', figure 1, data sequence 1 and 2, GEE $\chi^2 = 36.6$, $p < 0.001$, $B = -1.74 \pm 0.29$). Again, clutch sizes and egg masses did not differ between these rank 2 and rank 1 females (GEEs, $p = 0.76$ and 0.52, respectively).

Third, after removing their higher ranking female from the group, the medium females significantly increased the number of clutches produced as they rose from rank 2 to 1 (figure 1, medium females sequence 2 versus sequence 3, GEE $p < 0.001$, $B = -1.78 \pm 0.50$). Again, clutch size and egg mass did not change after these medium females changed rank (GEEs, $p = 0.68$ and 0.22, respectively). Note that also the small females changed rank (from rank 3 in sequence 2, to rank 2 in sequence 3). This rise in rank did not change the number of clutches produced (figure 1, GEE $p = 0.35$), but was accompanied by an increase in clutch size (GEE $p < 0.001$,

Table 1. Reproductive output of female group members depending on their rank, corrected for other effects. Coefficients are given for ranks 2 and 3 (rank 1 is the reference $B=0$) and sequence 2 (sequence 1 is the reference $B=0$). (Data sequences 1 and 2. GEE results with Wald χ^2, p-values and coefficients $B\pm$s.e., in all cases d.f.$=1$ except for rank d.f.$=2$. Body size, body size squared and body condition are covariates. Results are corrected for random individual effects.)

	number of clutches ($n=192$)[a]			clutch size ($n=136$)[b]			egg mass ($n=129$)[c]		
parameter	χ^2	p	$B\pm$s.e.	χ^2	p	$B\pm$s.e	χ^2	p	$B\pm$s.e.
intercept			-1.81 ± 0.83			2.25 ± 0.52			0.12 ± 0.20
rank [rank 2]	40.6	0.002	-1.53 ± 0.30	3.4	0.18	0.04 ± 0.23	4.5	0.10	0.03 ± 0.02
[rank 3]			-2.04 ± 0.37			-0.42 ± 0.26			0.06 ± 0.03
sequence [sequence 2]	1.9	0.17	-0.22 ± 0.16	0.6	0.45	0.09 ± 0.11	0.1	0.72	0.004 ± 0.01
body size (mm SL)	1.1	0.29	0.01 ± 0.009	15.9	<0.001	0.03 ± 0.01	3.1	0.08	0.01 ± 0.01
body size squared	—	—	—	—	—	—	3.0	0.09	-0.00009 ± 0.00005
body condition	5.7	0.017	0.64 ± 0.27	0.2	0.69	0.08 ± 0.21	0.8	0.39	-0.02 ± 0.02

[a] Sample from three females/group × two sequences × 32 groups = 192.
[b] Sample from 31 groups with data for 53 large females, 13 medium females and 10 small females.
[c] Sample from 31 groups with data for 50 large females, 13 medium females and 8 small females.

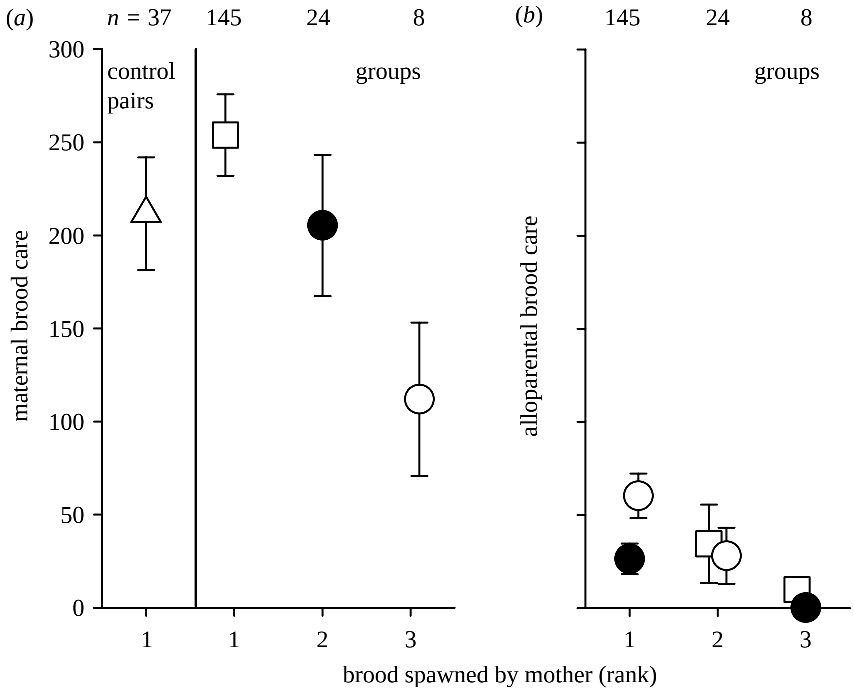

Figure 2. (*a*) Maternal- and (*b*) alloparental female brood care within control pairs (triangle) and groups of three females (ranked from the largest 1 to the smallest 3). Brood care by rank 1 females (squares), rank 2 females (filled circles) and rank 3 females (open circles). Means with standard errors are depicted with sample sizes (number of broods observed) indicated inside the graph.

$B=0.33\pm0.07$) and, maybe, egg mass (GEE $p=0.08$, $B=0.016\pm0.009$). However, sample sizes were very low for the small females (see figure 1). During sequence 3, the added very small females did not produce broods.

All of the females preferred to care for their own broods, i.e. maternal care was substantially higher than alloparental care for females of all ranks within groups (figure 2, GEE effect of maternity, d.f.$=1$, $p<0.001$). There was also a significant interaction between maternity and rank of the carer (GEE effect of rank carer, d.f.$=2$, $p=0.70$; effect of maternity × rank carer, d.f.$=2$, $p=0.043$), suggesting that alloparental care was not reciprocated equally. This was due to rank 1 females reducing their level of maternal brood care to the effort of lower-ranking females, but not vice versa, as follows: there was a significant negative correlation between rank 1 female maternal care, and both rank 2 female alloparental care ($r_p=-0.21$, $p=0.015$, $n=138$, partial correlation controlling for rank 3 female care) and rank 3 female alloparental care ($r_p=-0.22$, $p=0.01$, $n=138$, partial correlation controlling for rank 2 female care). No correlations were found between rank 2 female maternal care and alloparental care by the other females inside their group (versus rank 1 females: $r_p=-0.31$, $p=0.15$; versus rank 3 females: $r_p=-0.32$, $p=0.14$; both $n=21$). There were also no correlations between rank 3 female maternal care and alloparental care by the other females inside their group (versus rank 1 females: $r_p=-0.07$, $p=0.88$; versus rank 2 females: $r_p=-0.16$, $p=0.74$; both $n=5$).

4. DISCUSSION

The experiment shows that subordinate females in the cichlid *N. pulcher* are reproductively suppressed by more dominant females. The subordinates produced fewer clutches, of otherwise similar size and mass, compared with the dominant females of the same body size. Moreover, the subordinates gaining the dominant breeding position immediately increased their reproductive effort, matching those of control pairs.

It remains to be established whether suppression comes about by active interference of the dominant female in subordinate reproduction, by indirect interference or by commitment of the subordinates (see Hamilton 2004). Evidence for active interference has been found in this species. Dominant females eat subordinate eggs on the day of spawning and subordinates can only effectively circumvent this by securing an exclusive breeding patch (Heg & Hamilton 2008). Indirect interference may involve the dominants harassing the subordinates, which increases the subordinates' cortisol levels and through a cascade of other physiological effects may reduce the subordinates' reproductive potential (e.g. Young *et al.* 2006; see reviews Oliveira 2004; Adkins-Regan 2005). Analyses of stress-induced subordinate reproductive suppression via hormonal changes have given mixed results (e.g. Bennett *et al.* 1996; Creel & Waser 1997; Clarke *et al.* 2001; Schoech *et al.* 2004; Bender *et al.* 2006). Alternatively, some subordinates may simply not be interested in current reproduction to avoid eviction.

Dominant females may be selected to concede some reproduction to their female subordinates to acquire their continued help in brood care (see also Heg & Hamilton 2008). Indeed, the dominant females benefited from alloparental care, as they reduced their care behaviour concurrently ('load-lightening', Heinsohn 2004). Both the dominant and subordinate females showed far higher levels of maternal brood care than alloparental brood care; so future studies should include an unequivocal assessment of maternity to interpret brood care behaviour in this species.

The experiment was approved by LANAT of the Kanton Bern, and thus complied with the legal requirements of Switzerland.

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