

Female-mediated causes and consequences of status change in a social fish

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In highly social species, dominant individuals often monopolize reproduction, resulting in reproductive investment that is status dependent. Yet, for subordinates, who typically invest less in reproduction, social status can change and opportunities to ascend to dominant social positions are presented suddenly, requiring abrupt changes in behaviour and physiology. In this study, we examined male reproductive anatomy, physiology and behaviour following experimental manipulations of social status in the cooperatively breeding cichlid fish, *Neolamprologus pulcher*. This unusual fish species lives in permanent social groups composed of a dominant breeding pair and 1–20 subordinates that form a linear social dominance hierarchy. By removing male breeders, we created 18 breeding vacancies and thus provided an opportunity for subordinate males to ascend in status. Dominant females play an important role in regulating status change, as males successfully ascended to breeder status only when they were slightly larger than the female breeder in their social group. Ascending males rapidly assumed behavioural dominance, demonstrated elevated gonadal investment and androgen concentrations compared with males remaining socially subordinate. Interestingly, to increase gonadal investment ascending males appeared to temporarily restrain somatic growth. These results highlight the complex interactions between social status, reproductive physiology and group dynamics, and underscore a convergent pattern of reproductive investment among highly social, cooperative species.

Keywords: social status; dominance rank; cooperative breeding; testes size; cichlid fish;
Neolamprologus pulcher

1. INTRODUCTION

Cooperatively breeding vertebrates, as well as many social insects, live in social groups characterized by the formation of long-term dominance hierarchies, with frequent interactions between dominant and subordinate individuals. Such social living can promote the formation of status-dependent differences in behaviour, reproductive physiology and reproductive opportunities, with subordinates commonly exhibiting lower investment in gonads and reproductive hormone concentrations compared with dominants (Creel *et al.* 1992; Faulkes & Bennett 2001; Cant & English 2006; Clutton-Brock *et al.* 2006; Fitzpatrick *et al.* 2006; Desjardins *et al.* in press *a*). However, subordinates can and do ascend in social status by either inheriting their existing group, founding a new social group or assuming a breeding vacancy in a new social group (Monnin & Peeters 1999; Clutton-Brock *et al.* 2002; Buston 2003, 2004; Cant *et al.* 2006*a,b*; Stiver *et al.* 2006; Bridge & Field 2007). Yet, even when there are opportunities to ascend in status, some individuals refrain from doing so and thereby fail to reproduce. For example, in the Damaraland mole-rat (*Cryptomys damarensis*), non-reproductive subordinates avoid pairing with familiar or related breeding partners, presumably to avoid the costs of inbreeding (Jarvis & Bennett 1993; Clarke *et al.* 2001).

In general, the factors that influence social status change in cooperative societies are complex and varied, and to tease apart the causes and consequences of status change requires an experimental and integrative approach incorporating behaviour, physiology and genetic relatedness analyses.

Alterations in reproductive physiology following a change in social status have been investigated most extensively in non-cooperative species, and our understanding of the consequences of social status change is much more complete in these less social species. In non-cooperative species, increases in social status elicit rapid and dramatic responses, including increases in dominant social behaviours (Burmeister *et al.* 2005), gonadal growth (White *et al.* 2002), alterations in ejaculate characteristics, number and sperm allocation (Rudolfson *et al.* 2006; Cornwallis & Birkhead 2006, 2007; Pizzari *et al.* 2007) and elevated plasma androgen concentrations (Cardwell *et al.* 1996; Rudolfson *et al.* 2006). A small handful of studies have examined the interaction between reproductive physiology and increases in social status in cooperative vertebrates, and these have focused on endocrinological changes in newly promoted individuals (Faulkes & Abbott 1991; Clarke *et al.* 2001). Yet, in cooperative species, dominance hierarchies are far more stable, with dyadic interactions occurring frequently, probably leading to the formation of extreme status-dependent differences in reproductive physiologies.

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Table 1. Characteristics of control and experimental groups were similar prior to removals. (As male breeders can control more than one territory, removing a male breeder can provide a breeding vacancy in more than one social group. Five male breeders each controlled more than one social group, so while only 20 male breeders removed, 25 breeding vacancies were created. Data are mean $\pm$ s.e. (*n*); *t*-tests are used to compare control and experimental males.)

group characteristics	control	experimental	test	<i>p</i>
total group size (no. of individuals)	5.3 $\pm$ 0.6 (7)	6.3 $\pm$ 0.6 (18)	$t_{23}=1.03$	0.31
candidate male body length (mm)	52.7 $\pm$ 0.7 (7)	52.4 $\pm$ 0.2 (17)	$t_{22}=0.64$	0.53
male breeder body mass (g)	6.1 $\pm$ 0.1 (5)	6.1 $\pm$ 0.1 (15)	$t_{18}=0.41$	0.69
male breeder standard length (mm)	55.6 $\pm$ 3.0 (5)	57.6 $\pm$ 1.5 (15)	$t_{18}=0.61$	0.55
female breeder body mass (g)	3.7 $\pm$ 0.2 (7)	3.6 $\pm$ 0.1 (18)	$t_{23}=0.22$	0.83
female breeder body length (mm)	52.6 $\pm$ 1.2 (7)	52.3 $\pm$ 0.6 (18)	$t_{23}=0.24$	0.82

In this study, we examined the relationship between social status and male reproductive behaviour, physiology and anatomy in the cooperatively breeding cichlid fish *Neolamprologus pulcher*, endemic to Lake Tanganyika, Africa where they live permanently in social groups. We created breeding vacancies by experimentally removing dominant male breeders, thus offering large male helpers the opportunity to ascend in social status. We predicted that ascending males would behave more aggressively and would invest more in gonadal tissues and produce more reproductive hormones. We also examined the social context in which social status changes did and did not occur in relation to size differences among group members, social dynamics and the genetic relatedness between male helpers and dominant female breeders.

2. MATERIAL AND METHODS

(a) *Study species*

Neolamprologus pulcher lives in permanent social groups composed of a dominant breeding pair and subordinate male and female helpers that assist in territory defence and maintenance, as well as in brood care (Taborsky & Limberger 1981; Balshine-Earn *et al.* 1998; Balshine *et al.* 2001a; Heg *et al.* 2005). Average relatedness between breeders and helpers is low (less than that of second-degree relatives; Stiver *et al.* 2005) and, as a result of frequent breeder turnover (Stiver *et al.* 2004), large helpers tend to be less related to the breeding pair than small helpers (Dierkes *et al.* 2005). Dominant male breeders have much larger testes and higher androgen levels than subordinate male helpers of similar size (Fitzpatrick *et al.* 2006; Desjardins *et al.* in press a). As larger testes produce and store more sperm (Møller 1988, 1989; Schärer *et al.* 2004), and testes size is positively correlated with reproductive success (Awata *et al.* 2006), the differences in testis size between dominants and subordinates suggest that helpers are unlikely to successfully sire many offspring in natural populations (but see Dierkes *et al.* 1999; Heg *et al.* 2006 for evidence that male helpers gain paternity in laboratory populations).

(b) *Experimental protocol*

This study was conducted from 26 February to 26 April 2005 at depths of 10–13 m in Kasakalawe Bay, Lake Tanganyika, Africa (8°46' S, 31°46' E; see Balshine *et al.* 2001a; Stiver *et al.* 2005; Fitzpatrick *et al.* 2006). We surveyed the study site using SCUBA, locating 25 groups in which there was a large male helper. In our study population, 43% of groups had large (greater than 50 mm) helpers; 27% of groups had a large male helper and 16% had a large female helper

(J. L. Fitzpatrick, J. K. Desjardins, K. A. Stiver & S. Balshine 2004, 2005, unpublished data). We called these large male helpers 'candidate males' because they fell within the 95% CI of standard lengths (SLs) of male breeders in our study population (see Stiver *et al.* 2006). Using a 5 $\times$ 1 m² fence net, we captured, sexed (by examining the genital papilla), measured (SL to the nearest mm) and individually marked fish using a non-toxic latex paint (Balshine *et al.* 2001a). Fish were not harmed during marking and quickly resumed normal behaviours upon release back into their social group.

Of the 25 groups with candidate males, we permanently removed the breeding male in 18 experimental groups, while in 7 control groups we held the breeding male in a hand net for 15 min, then returned him to his social group. Hence, candidate males in control groups (control males) did not experience an opportunity to ascend in social status. Not all candidate males in the experimental groups ascended in social status: in nine of these groups, the candidate males assumed the breeding vacancy following male breeder removal (we called these 'ascending males'), while in the other nine groups, a male breeder from a neighbouring territory assumed the breeding vacancy and the candidate males in that group remained socially subordinate (we called these 'non-ascending males'). Thus, the experiment created three types of candidate male: ascending (*n*=9), nonascending (*n*=9) and control males (*n*=7). Prior to removals, there were no significant differences among the different types of candidate male in body size, group characteristics (table 1) or behaviours (feeding, aggression given/received and submission given/received (all *p* > 0.14).

(c) *Behavioural observations*

Male breeders were observed during two 7-min focal watches on the day of their removal (day 0). Candidate males were observed for two 7-min focal watches prior to removal of the breeder male (day 0) and on 2 days after the breeder removal (days 1 and 6). On each of these 3 days, in each group we conducted two focal watches on the candidate male helper, the female breeder and any new male to arrive in the group: watches were conducted once in the morning (9.00–12.00 local time, GMT +2) and once in the afternoon (12.00–18.00), to control for diurnal variation in behaviours (Werner *et al.* 2003). Data from the two watches for each fish on a given day were averaged to yield a single behavioural score for that day. Recording data on PVC slates, we noted the frequency of feeding, aggressive acts (rams, chases, threat displays, bites and mouth fights) and submissive acts (tail quivers and submissive postures) directed towards, or received from, group members. To assess social dominance, we calculated a

dominance index as (aggressive acts given + submissive acts received) – (aggressive acts received + submissive acts given) during focal watches (adapted from White *et al.* 2002).

(d) Fish collection

On day 7, the day after the final focal watch, candidate males and female breeders were collected from each group using a conical tent net and a small volume (3–7 ml) of the anaesthetic quinaldine (2-methylquinoline; $C_6H_4N : C(CH_3)CH : CH$; see Fitzpatrick *et al.* 2006). On shore, fish were killed with a lethal dose of anaesthetic (benzocaine; ethyl *p*-aminobenzoate) and their body mass and gonad mass were measured to the nearest 0.001 g. SL was measured to the nearest 0.1 mm. Note that SL of candidate males was measured both before and after the experiment. On day 7, SL was measured by a researcher blind to pre-experimental body measurements. Blood and fin tissue samples were taken for hormonal and genetic analyses, respectively, and sex was confirmed by dissection. Testes were removed, weighed and placed on a clean, dry microscope slide for sperm extraction.

(e) Testes and sperm analyses

To assess the relative gonadal investment of ascending males, we compared their relative testes mass (controlling for body mass) with that of 16 established male breeders each holding a single territory. In our study population, male breeders controlled one to six territories, each territory containing its own female and helpers. Gonadal investment can vary with the number of territories held (Desjardins *et al.* in press b). Since ascending males were dominant in only one territory, single territory holding males, collected from the same subpopulations, were used to compare gonadal investment. Importantly, males holding a single territory and the candidate males of all types had similar group sizes (ANOVA $F_{2,40} = 1.21$, $p = 0.31$).

Sperm swimming speed was measured following Fitzpatrick *et al.* (2006). Briefly, a Leica DME light microscope (Leica Microsystems Inc., Buffalo, NY, USA) mounted with a PixeLINK Megapixel PL-A662 digital video camera (PixeLINK, Ottawa, Ontario, Canada) was used to record sperm motility. Video recordings were captured at 60 frames s^{-1} at 200 $\times$ magnification, starting when milt was diluted with water. Sperm swimming speed (VAP) was measured for 1 s at 30 s and 60 s post-activation, then at 1-min intervals until 9 min after activation. We used a CEROS (v. 12) computer-assisted sperm analysis system (Hamilton-Thorne Research, Beverly, ME, USA) to measure the swimming speed of all spermatozoa whose forward movement was recorded for more than or equal to 20 frames (mean number of sperm recorded $\pm$ s.e.: 18 ± 0.9 ; range: 5–103; see Fitzpatrick *et al.* 2006 for further details).

Sperm lengths were measured using milt (sperm and seminal plasma) flowing from dissected testes, diluted with lake water and allowed to air dry on a glass slide. Ten clearly visible sperm from each male were photographed and measured to the nearest 0.1 μm (magnified 1000 $\times$) using NIH IMAGEJ software (v. 1.38, available at <http://rsb.info.nih.gov/ij/>) by tracing a freehand line from the centre of the head to the end of the tail (see Balshine *et al.* 2001b) using an Intuos graphic tablet (Wacom Co. Ltd., Japan). All sperm lengths were measured blind to the identity of males.

(f) Hormonal analyses

A blood sample was collected for hormone analysis by caudal severance using heparinized micro-capillary tubes. Blood was spun at 14 000g for 10 min, separating blood plasma from red blood cells. Plasma was stored at $-10^\circ C$ until 11-ketotestosterone (11KT) concentrations, a primary fish androgen, could be assessed using radioimmunoassay (see Desjardins *et al.* 2006 for further details).

(g) Genetic analysis

Using DNA extracted from muscle tissue, individuals were genotyped at 12 microsatellite loci optimized for *N. pulcher* (see Stiver *et al.* 2005, 2007, in press for further details). Individuals were successfully genotyped at an average of 9 loci (range: 4–12 loci). Relatedness (Queller's r) between individuals was estimated using the program KINSHIP 1.3.1 (Goodnight & Queller 1999).

(h) Statistical analyses

Statistical analyses were performed using JMP (v. 6.0.3, SAS Institute, Inc. 2006). All descriptive statistics are presented as mean $\pm$ s.e. Data were transformed whenever necessary to improve the fit to normality. Whenever the assumptions of normality and equal variances could not be met, non-parametric statistics were applied. Behavioural data (individual behaviours and dominance index scores) were analysed using ANOVAs on ranks (Zar 1999). Testes mass (log transformed) was compared among male types using ANCOVA with body mass (log transformed) as a covariate. Whenever main effects were significant, Tukey's *post hoc* tests were used to identify significant differences between male types. We confined our genetic and hormonal analyses to only those male types (ascending and non-ascending) that had an opportunity to ascend in status (i.e. control males that had no opportunity to ascend were not included in the analysis). Note that hormonal information was not successfully obtained from every candidate male, thus reducing our sample sizes for these statistical analyses.

3. RESULTS

(a) How did status change influence male behaviour?

Prior to the removals (day 0), the original male breeders performed significantly more dominant behaviours than non-ascending and control males (ANOVA on ranks, $F_{3,41} = 5.77$, $p = 0.002$) and all helpers had similar dominance indices (figure 1). Ascending males performed more dominant behaviours and had significantly higher dominance index scores on day 1 following breeder removals compared with non-ascending and control males ($F_{2,21} = 6.72$, $p = 0.006$, figure 1). These behavioural differences persisted on day 6 ($F_{2,22} = 8.37$, $p = 0.002$). Following removals, ascending males performed more dominant behaviours, with similar dominance index scores to those of the original male breeders (day 1: $F_{1,27} = 0.01$, $p = 0.92$; day 6: $F_{1,27} = 0.11$, $p = 0.91$, figure 1). Ascending males also received significantly more submissive behaviours from the female breeders (day 1: $F_{2,21} = 12.85$, $p = 0.0002$; day 6: $F_{2,22} = 5.56$, $p = 0.01$) than did non-ascending and control males, indicating that ascending males had indeed become socially dominant in their group. The mean frequency of feeding did not differ among male

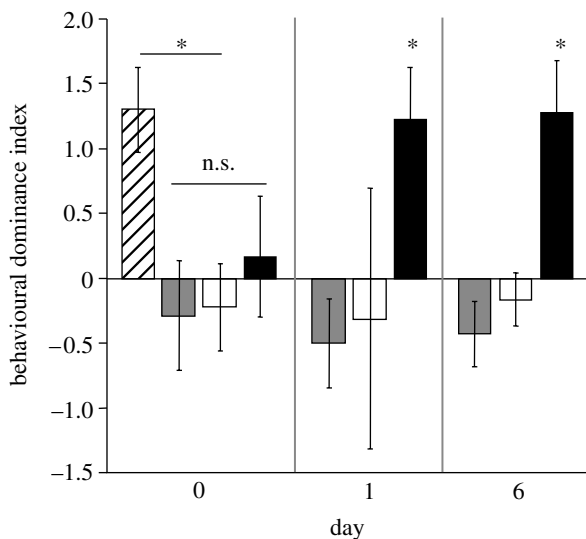


Figure 1. Mean behavioural dominance index score ($\pm$ s.e.) in original male breeders prior to removal (day 0, $n=20$, hatched bars), and in control ($n=7$, grey bars), non-ascending ($n=9$, white bars) and ascending males ($n=9$, black bars) before (day 0) and after (days 1 and 6) male breeder removals. Positive values indicate that more dominant behaviours were performed than subordinate behaviours. Tukey's *post hoc* tests revealed that ascending males scored significantly higher on the dominance index than both control and non-ascending males on days 1 and 6, but control and non-ascending males did not differ on any day. Asterisks indicate significant differences between male types and n.s. indicates non-significant differences.

types (control, ascending and non-ascending) following removals (repeated measures ANOVA, effect of male type, $F_{2,22}=0.17$, $p=0.84$).

(b) How did status change influence male reproductive physiology?

One week after removals, ascending males testes masses were similar to those of the removed male breeders (controlling for body size) and were significantly larger than the testes of subordinate males (non-ascending and control males; ANCOVA, effect of male type, controlling for body mass: $F_{3,36}=5.75$, $p=0.003$, figure 2). Ascending males had testes that were 1.66 and 1.72 as heavy as those of control and non-ascending males, respectively. Controlling for body size, testes mass from control males that were larger than their female breeder ($n=3$) did not differ from those of control males that were smaller than their female breeder ($n=4$, *t*-test, $t=-0.004$, $p=1.0$).

Sperm swimming speeds (VAP) from all four male types (including male breeders) were similar (repeated measures ANOVA: $F_{3,29}=1.36$, $p=0.28$). Median sperm tail length also did not differ significantly among male types ($F_{3,30}=1.07$, $p=0.38$).

Testes mass was negatively correlated with the change in SL of ascending males ($r=-0.72$, $p=0.03$, $n=9$; figure 3a), suggesting that males who grew larger testes did so at the expense of somatic growth. There was no such trade-off among non-ascending ($r=-0.03$, $p=0.94$, $n=8$; figure 3b) or control males ($r=-0.23$, $p=0.62$, $n=7$; figure 3c). Following breeder removals, there was no significant difference among male types in growth measured as the change in SL ($F_{2,21}=1.28$, $p=0.30$).

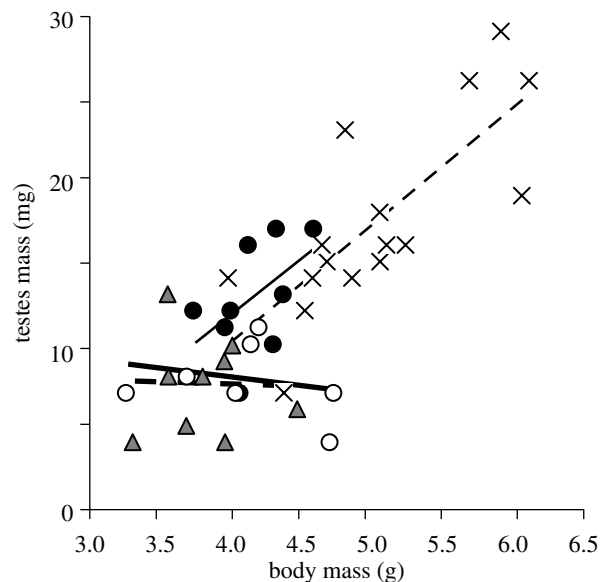


Figure 2. Relations between testes mass and body mass of control (triangles and thick dashed line), non-ascending (open circles and thick solid line) and ascending candidate males (closed circles and thin solid line), as well as in previously established male breeders (crosses and thin dashed line). Tukey's *post hoc* tests showed that ascending males and male breeders did not differ significantly in testes mass 7 days after breeder male removals, but invested significantly more in gonads than either non-ascending or control males (who did not differ significantly from each other).

Mean 11KT levels in ascending males (1301.1 ± 443.8 pg ml $^{-1}$, $n=7$) were 4.7 times those observed in nonascending males (275.9 ± 81.6 pg ml $^{-1}$, $n=3$), but this difference was not significant (Wilcoxon–Mann–Whitney test, $S=2.92$, $p=0.09$). Considering ascending and non-ascending males together, there was no relationship between 11KT and testes mass (Spearman's rank correlation, $r_s=0.32$, $n=10$, $p=0.37$). Mean 11KT levels from control males that were larger than their female breeder ($n=2$) did not differ from those of control males that were smaller than their female breeder ($n=4$, $S=0.86$, $p=0.35$).

(c) What factors influenced the probability of ascension?

In this section, we confined our analysis to only those male types (ascending and non-ascending) that had an opportunity to ascend in status. Male ascension success was significantly predicted by four factors: initial candidate male body size, female breeder body size, the body size ratio between candidate males and their female breeders, and the relative size differences between candidate males and their female breeders (logistic regressions, all $p \leq 0.05$). A stepwise logistic regression with all four variables as potential predictor variables showed that the size differences between the candidate male and their female breeder was the only significant factor influencing the probability of male ascension, ($\chi^2=12.58$, $p=0.0004$, $n=18$, $R^2=0.53$; figure 4). In all cases of ascension, ascending males were larger than the female breeder in their group, while six out of eight males who did not ascend in social status were smaller than their female breeder.

Pairwise relatedness estimates between candidate males and the female breeder did not differ significantly between ascending ($n=9$, $r=0.001 \pm 0.09$, range = -0.30 to 0.56)

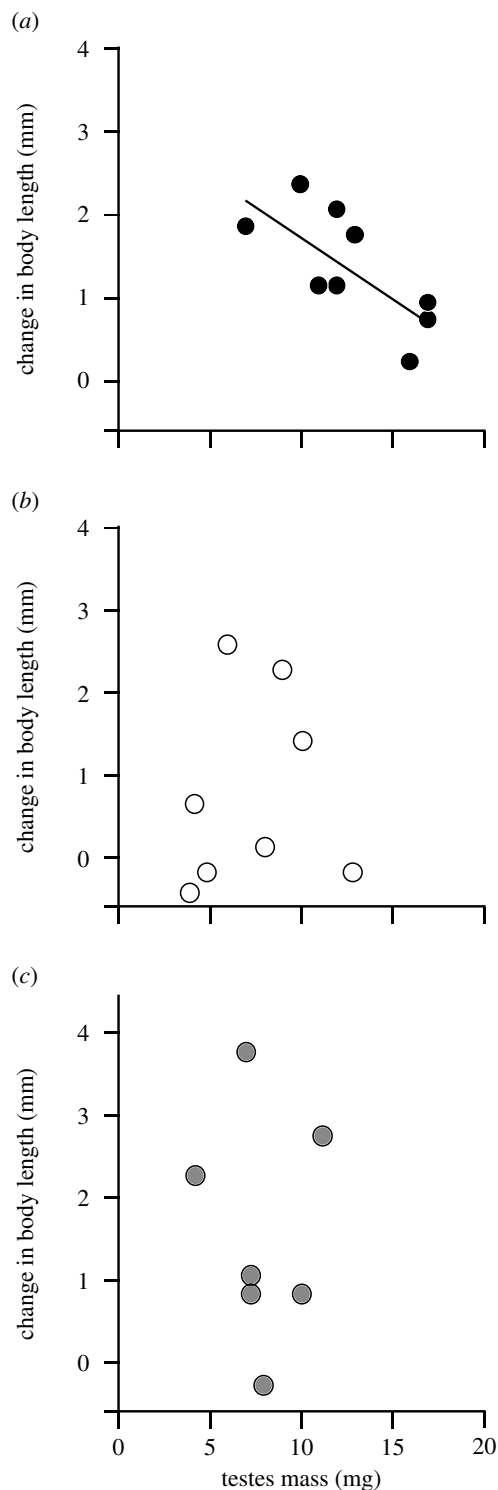


Figure 3. Relations between testes mass and the change in body length (SL) of candidate males over the 7-day period following breeder removal in (a) ascending, (b) non-ascending, and (c) control males.

and non-ascending ($n=9$, $r=0.11 \pm 0.12$, range = -0.34 to 0.71) males following male breeder removal ($t_8=0.77$, $p=0.45$).

4. DISCUSSION

In an ever-changing social landscape, where breeding opportunities can arise suddenly, subordinates need to adapt quickly. In this study, we demonstrate how

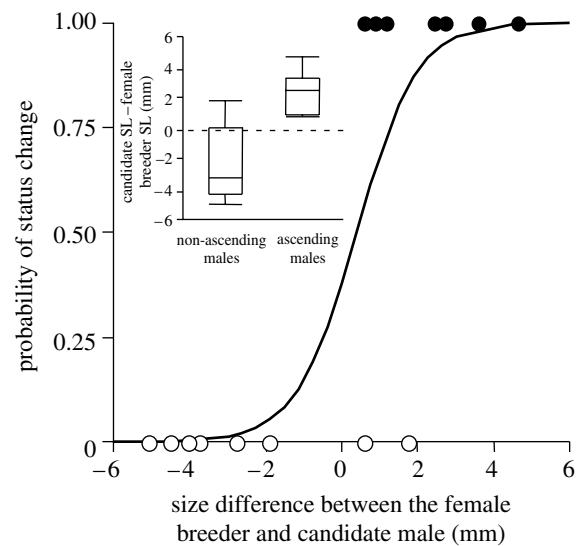


Figure 4. Logistic regression showing the probability of a candidate male ascending in social status in relation to the initial difference in size (SL) between that male and the female breeder in his social group. Filled circles indicate ascending males and open circles indicate males that did not ascend. In the inset of the figure a box plot shows the 10th, 25th, 50th (median), 75th and 90th percentiles of the size difference between males and female breeders in ascending and non-ascending groups.

immediate changes in social status are associated with rapid changes in behaviour, anatomy and reproductive physiology. Following our experimental removals, ascending males performed more dominant behaviours, which seems likely to have served to secure the dominant social position. In social insects and mammals, individuals newly ascended to dominant positions commonly perform more aggressive behaviours towards subordinates, but once a new social order is established, levels of aggression subside (Sapolsky 1983; Clarke & Faulkes 1997; Monnin & Peeters 1999; Cant *et al.* 2006a). High levels of sustained aggression may not be necessary to maintain a dominance hierarchy, particularly if subordinates engage in 'peaceful cooperation' by refraining from challenging the dominant individuals (Buston 2004b; Buston & Cant 2006; Wong *et al.* 2007; Buston & Balshine 2007). However, in contrast to many other highly social species, in our study, as in studies of other social fishes (Fricke & Fricke 1977; Booth 1995), the level of aggression did not subside following the establishment of the new social order, suggesting that high levels of aggression are required to maintain social dominance hierarchies in *N. pulcher*.

In addition to our behavioural results, we also confirmed that increases in testicular mass were associated with social status change in a cooperative vertebrate. Previous studies have documented dramatic increases in gonadal tissue and circulating reproductive hormone concentrations (e.g. Cardwell *et al.* 1996; White *et al.* 2002; Rudolfson *et al.* 2006) as well as rapid reorganization of gonadal tissue resulting in sex change (e.g. Robertson 1972) following increases in status in non-cooperative species. In *N. pulcher*, ascending males tended to have elevated 11KT concentrations, but we were limited by small sample sizes and future work should focus on more fully exploring the relation between status change and reproductive hormone levels. Also, we cannot rule out the possibility that ascending

males may have initially been physiologically different from non-ascending and control males, as ascending males had greater testes masses by the end of the experiment. We have previously shown that large male helpers (of our candidate male size range) invest far less in testes mass (Fitzpatrick *et al.* 2006) and have low levels of circulating reproductive hormones compared with breeders (Desjardins *et al.* *in press a*), suggesting that, prior to status change, candidate males in our study had similar reproductive physiologies. Furthermore, in the control treatment, where the social hierarchy remained intact, testicular investment and 11KT concentrations were not influenced by the relative size difference between candidate males and their female breeder, again supporting the notion that the observed alterations in reproductive physiologies were a consequence of status change. The reduced reproductive investment implies that extensive reproduction by subordinate males is unlikely in natural *N. pulcher* populations (but see Dierkes *et al.* 1999; Heg *et al.* 2006 for laboratory support to the contrary). To increase reproductive output, alterations in the reproductive physiology of newly promoted males are essential. Thus, we propose that the physiological changes observed in ascending males were the result, rather than the cause, of social status change, although verification of this hypothesis would require manipulation of helper physiology before breeding vacancies were presented.

The observed trade-off between somatic and gonadal growth may serve to limit the probability of successful ascension. Most males that achieve dominance in a social group, and thus breeding status, have only a few opportunities to breed, as breeder turnover is frequent (Stiver *et al.* 2004) and these males are severely reproductively limited by females who lay eggs only once each month following the lunar cycle (Balshine *et al.* 2001a). As social dominance in *N. pulcher* is primarily determined by body size, males presented with an opportunity to ascend in social status must be large enough to socially dominate the female breeder in their group, while rapidly mobilizing resources towards reproduction and gonadal growth to capitalize on breeding opportunities when they become available. Indeed, only male helpers that were larger than the female breeder were able to secure the dominant social position within a group. Thus, we propose that social ascension will occur only when male helpers are larger than (i) a size threshold relative to neighbouring male size, where candidate males are large enough to successfully compete with neighbouring males and (ii) a relative size threshold between the candidate male helper and the female breeder, such that ascending males can immediately behaviourally dominate the female breeder and begin to invest in testes mass. Both these thresholds will not be based on absolute body size but on a dynamic interplay between the ascending male's size and the sizes of the fish it will interact with (both the potential new mate and competitors). Thus, in *N. pulcher*, status change is not influenced by male–male interactions alone, but the female breeder plays a unique role in influencing the success of male status change. Once an ascending male establishes social dominance in his single group, and begins to achieve reproductive success, resources again may shift from investment in gonadal growth back to investment in somatic growth in order to secure additional breeding positions (by taking over other territories).

Despite the relation between testicular mass and social status in *N. pulcher*, changes in the social status of males were not associated with changes in either the morphology or swimming speed of their spermatozoa. Yet, in non-cooperative species, social status has been found to influence sperm function, with subordinate males exhibiting impaired sperm motility when they are suppressed by a dominant (Koyoma & Kamimura 1999, 2003; Hermes *et al.* 2005), or enhanced sperm motility when they attempt to parasitize the reproduction of the dominant male and their ejaculates are subject to sperm competition (Froman *et al.* 2002; Rudolfsen *et al.* 2006). In contrast, in highly social African mole-rats (genera *Cryptomys* and *Heterocephalus*), gonadal investment and reproductive hormone concentrations were higher in dominant reproductive males than non-reproductive subordinate males but, as in our study, non-reproductive males produced sperm that swam at speeds similar to those of reproductive males (Faulkes & Abbott 1991; Faulkes *et al.* 1991, 1994; Maswanganye *et al.* 1999; Faulkes & Bennett 2001; van Rensburg *et al.* 2003). Given that subordinate males in both *N. pulcher* and some African mole-rats have smaller testes, fewer motile sperm and low concentrations of androgens compared with dominant males, why are sperm characters similar between males of different social status? We argue that, in cooperative breeders, constant social interaction with group members selects for reduced investment in testes mass and androgens in subordinates in order to avoid the high costs of group expulsion (Balshine-Earn *et al.* 1998). We also argue that the constrained and temporally uncertain opportunities to breed select for functional sperm maintenance in subordinates as it facilitates immediate reproductive success following ascension to a dominant social position (see van Rensburg *et al.* 2003 for a similar argument).

Unlike most other species, cooperative breeders live in social groups for their entire lives, have severe limitations on breeding opportunities, and must seize any reproductive opportunity instantly if they are to achieve any measure of reproductive success. Constant and repetitive social interactions in cooperatively breeding species may habituate subordinates to the effects of social stress (Creel 2001) and facilitate the production of viable sperm, albeit in small testes. We argue that the unpredictable and rapidly changing social landscape experienced by most cooperative breeders contrasts with the usual temporally limited breeding seasons experienced by most other organisms. When individuals can mobilize and organize their reproductive machinery in a predictable manner (e.g. based on seasonality of reproductive cues), they can either upregulate (if sneaking) or downregulate (if socially subordinate for a predictable time frame, such as a breeding season) sperm physiologies in relation to their predictable social status. The present study considerably broadens the idea that reproductive investment is flexible and can change rapidly in highly social species. We also describe the unique role played by dominant females in influencing the probability of male status change. Our results highlight a convergent pattern of reproductive investment among highly social, cooperative species, and illustrate the need for integrative studies when examining male reproductive physiology.

Research protocols described in this study were approved by the Animal Research Ethics Board of McMaster University and adhere to the Canadian Council of Animal Care guidelines. This research was conducted with the support, cooperation and permission of the Zambian Department of Fisheries.

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