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Male reproductive suppression in the cooperatively breeding fish *Neolamprologus pulcher*

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In most cooperative breeders, dominants suppress the reproduction of subordinates. However, two previous studies of *Neolamprologus pulcher*, a cooperatively breeding cichlid fish, have suggested that socially subordinate helper males sneak fertilizations from dominant breeding males. If such sneaking does occur, both theoretical work and empirical studies of other fish species suggest that sperm competition will select for increased reproductive investment by sneaker males, relative to more dominant males. To address these issues, we quantified gonadal investment and sperm characteristics of 41 *N. pulcher* male breeders and 62 male helpers from 55 groups in Lake Tanganyika. Gonadal investment followed patterns consistent with reproductive suppression, with breeders having considerably larger testes masses than helpers. Breeders also had faster and longer swimming sperm and a higher percentage of motile sperm compared to helpers. However, sperm characteristics of large helpers were similar to those of breeders, but these same helpers had lower testes masses. Thus, large helpers had sperm that were physiologically equivalent to that of breeders, but their relatively small gonads imply that they were reproductively suppressed. *Key words:* Cichlidae, dominance hierarchies, Lake Tanganyika, reproductive physiology, social status, sperm competition. *[Behav Ecol 17:25–33 (2006)]*

Reproductive suppression—the limitation of reproduction in sexually mature, socially subordinate individuals by more dominant group members—is common in many cooperatively breeding animals (for example, bell miners, *Manorina melanophrys*, Poiani and Fletcher [1994], dwarf mongooses, *Helogale parvula*, Creel and Waser [1997], both, Damaraland, *Cryptomys damarensis*, and naked mole-rats, *Heterocephalus glaber*, Faulkes and Bennett [2001], and meerkats, *Suricata suricatta*, O'Riain et al. [2000]; Griffin et al., 2003). Reproductive suppression may be enforced by behavioral and/or chemical means (Moehlman and Hofer, 1997), with physiological consequences ranging from reductions in reproductive hormone levels (Bennett et al., 1997, 2000; Faulkes and Abbott, 1997; Faulkes and Bennett, 2001) to impaired or delayed gonadal and gametic development (Faulkes et al., 1994; Maswanganye et al., 1999).

In cooperatively breeding species, one way a dominant individual can monopolize reproduction is by suppressing a subordinate's reproductive capacity. The degree of monopolization, ranging from low levels in plural breeders to high levels in singular breeders (Mumme, 1997), is influenced by various ecologic, genetic, and social constraints. Reproductive monopolization is expected to be high if relatedness between dominant and subordinate individuals is high (i.e., due to inbreeding avoidance; Pusey and Wolf, 1996), if ecological constraints on independent dispersal and breeding are high, and/or if the fighting ability of dominant individuals easily surpasses that of subordinates (Heg et al., 2004a; Keller and Reeve, 1994; Reeve et al., 1997). As ecologic and genetic constraints are relaxed, dominant individuals may concede a larger degree of reproduction to subordinates in order to entice subordinates to stay in the group (Clutton-Brock, 1998; Heg et al., 2004a; Reeve et al., 1997). Alternatively, under these conditions subordinates may claim a larger degree of reproduction for themselves as the costs of expulsion from the group diminish (Reeve et al., 1997).

In this study, we examined the relative gonadal investment and gametic characteristics of dominant breeders and subordinate helpers in the cooperatively breeding cichlid, *Neolamprologus pulcher*. *N. pulcher*, endemic to Lake Tanganyika, Africa, lives in social groups consisting of a breeding pair and 1–15 male and female helpers that assist in territory defense, territory maintenance, and brood care, while enhancing offspring survival within a group (Balshine et al., 2001; Brouwer et al., 2005; Taborsky, 1984). Breeding pairs are faithful to a territory, and within each territory, a strict dominance hierarchy is maintained, with breeders dominant to all helpers (Taborsky, 1984). Behavioral observations suggest that only the breeding pair in each group reproduce (Balshine et al., 2001; Balshine-Earn et al., 1998). Under this social system, as in many cooperatively breeding mammals and birds, subordinate individuals are likely to be reproductively suppressed, and consequently have smaller gonads. However, two recent studies suggest that sperm competition between breeders and helpers may in fact favor (select for) larger gonadal and gametic investment by helpers (Dierkes et al., 1999; Dierkes P, Taborsky M, and Achmann R, personal communication), opposite to the expected pattern if reproductive suppression is operating in this system.

Sperm competition occurs when sperm from different males compete over access to a single female's eggs. Traits that provide males with a selective advantage for such competition will be favored and can evolve when two or more males

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regularly compete to fertilize eggs (Parker, 1970). For example, larger testes or faster swimming sperm may provide males with a competitive advantage (Ball and Parker, 1996; Parker, 1990a,b, 1993). In a laboratory study, Dierkes et al. (1999) demonstrated that large, subordinate *N. pulcher* males engaged in sneak fertilizations and successfully fathered between 12.5% and 35.8% of the young. In a complementary field study, mixed paternity was found, but sampled subordinate male helpers from those groups were not the fathers; however, the researchers argued that the subordinate males did successfully sneak fertilizations and father young and were subsequently expelled from the group (Dierkes P, Taborsky M, and Achmann R, personal communication). Hence, it remains unclear whether helper males engage in sneak fertilizations in the wild.

Reproductive suppression and sperm competition should select for different, and opposing, levels of gonadal/gametic investment in breeders and helpers, providing testable predictions regarding an individual's reproductive investment. Reproductive suppression typically results in males in the disadvantaged role (physically subordinate or sneaking males) investing *less* in gonads and gametes (Johnstone and Cant, 1999). The three studies performed to date describing reproductive investment in cooperatively breeding species provide empirical support for these predictions. In the naked mole-rat, sperm from nonbreeding males were less concentrated in the ejaculate and exhibited impaired motility (Faulkes et al., 1994), and adult-sized, nonbreeding females had reproductive tracts in a prepubescent state of development (Faulkes and Abbott, 1997). Similarly, nonbreeding male Damaraland mole-rats had a greater proportion of immature sperm in their ejaculate relative to breeding males in the same social group (Maswanganye et al., 1999), but in this system helpers do not invest in gonads and gametes due to the costs associated with inbreeding rather than active reproductive suppression by dominant breeders (Bennett et al., 1996). Finally, in the dwarf mongoose, subordinate males had smaller gonad sizes relative to dominant male breeders (Creel et al., 1992).

In contrast, with sperm competition, disadvantaged males (physically subordinate or sneaking males) are expected to invest relatively *more* energy in gonads and gametes than do dominant males (Parker, 1990b). Both theoretical and empirical studies demonstrate that sperm competition can select for different, but adaptive, levels of gonadal investment by males of the same species (Taborsky, 1994, 1998, 2001). In several fish species where some males are known to engage in sneak fertilizations, sneakers invest relatively more resources in their gonads than do dominant individuals, including salmonids (Gage et al., 1995; Vladic and Järvi, 2001), bluegill (*Lepomis macrochirus*; Gross and Charnov, 1980; Leach and Montgomerie, 2000), bluehead (*Thalassoma bifasciatum*) and saddleback wrasses (*Thalassoma duperrey*; Henson and Warner, 1997; Ross et al., 1983; Warner, 1982), plainfin midshipman (*Porichthys notatus*; Brantley and Bass, 1994; Foran and Bass, 1998; Grober et al., 1994), and platyfish (*Xiphophorus maculatus*; Halpern-Sebold et al., 1986), to mention only a few of the well-studied species.

Sperm swimming velocity and duration have often been used as indices of sperm quality. Recently, studies examining sperm characteristics in a variety of species have demonstrated a positive relationship between sperm swimming velocity and fertilization success (Cieresko and Dabrowski, 1994; Gage et al., 2004; Lahnsteiner et al., 1998; Levitan, 2000; Malo et al., 2005; Moccia and Munkittrick, 1987) and a negative relation between the duration of sperm motility and fertilization success (Ginsburg, 1963; Hoysak and Liley, 2001). To date, sperm characteristics of sneaker males in relation to territorial (or parental) male fishes have only been examined in Atlantic salmon (Gage et al., 1995; Vladic and Järvi, 2001; Vladic et al., 2002) and bluegill (Burness et al., 2004; Leach and Montgomerie, 2000). In salmon, sperm from sneaker males had a greater percentage of motile spermatozoa and greater energy stores (Gage et al., 1995; Vladic and Järvi, 2001) but no differences in sperm velocity, duration of motility, or morphology compared to dominant males (Vladic and Järvi, 2001; Vladic et al., 2002). In bluegill, sperm from sneaker males initially swam faster, had greater energy stores (Burness et al., 2004), and was 50% more concentrated in ejaculates (Leach and Montgomerie, 2000) than the sperm of parental males.

In this study, we examine the gonadal investment and gametic characteristics of *N. pulcher*, comparing our results to predictions from reproductive suppression and sperm competition theory. This is the first study to offer a detailed analysis of sperm characteristics in a nonmammalian cooperative breeder and only the third examination of sperm characteristics in a cooperatively breeding animal (the other two were on mole-rats: the naked mole-rat, Faulkes and Abbott, 1991; Faulkes et al., 1994; Jarvis, 1991, and Damaraland mole-rat, Maswanganye et al., 1999).

METHODS

We studied 55 *N. pulcher* groups between 5 February and 15 April 2004 in Kasakalawe Bay, Lake Tanganyika (8° 46′ S; 31° 46′ E). Groups were studied at depths of 10–13 m using SCUBA. After an initial habituation period (approximately 3–5 min) for each group, group compositions and dominance hierarchies were ascertained in two to four separate observational sessions. Groups were visited three to eight times throughout the course of this study. In each group, 10-min focal watches were also conducted on the breeding pair and the two largest helpers, and the number of aggressive (ramming, chasing, biting, mouth fighting) and submissive (tail quivers, submissive postures) acts performed by each focal fish was recorded. Two watches were conducted on each individual. On each visit to a territory, group size was recorded. Social dominance was assigned using an index that combined behavioral observations, relative body size, and body color markings (see Buchner et al., 2004). Helpers were categorized by their position in the dominance hierarchy, as shown in Table 1. Between groups, an individual's position within this dominance hierarchy did not necessarily correlate with its absolute size because the largest helpers (helper 1) in any one group may have actually been quite small. Conversely, helpers further down in the dominance hierarchy could be relatively large if there were several large helpers in the group. Fish were individually identified based on territory affiliation, estimates of body length, natural body markings, and experimental markings with a nontoxic latex paint (see Balshine et al., 2001 for further details). All data were recorded on PVC slates.

Table 1

Sample sizes and body size ranges for the gonad and sperm data analyzed from breeder and helper male *Neolamprologus pulcher*

Social status	*n*	Size range (mm)
Breeding males	41	66–49
All helper males	62	57–24
Helper 1	25	57–29
Helper 2	12	50–22
Helper 3	6	46–31
Helper 4	5	44–26
Helper 5	10	42–24
Helper 6	4	34–27

After the final observation period, we attempted to collect all the individuals in each group by placing a conical tent net, equipped with weights at the bottom and a float at the top, directly over the territory and brood chamber area and securing the net perimeter to the substrate with rocks (see Morley and Balshine, 2002 for further details). A small volume (3–7 ml) of quinaldine [2-methylquinoline; $C_6H_4N{:}C(CH_3)CH{:}CH$)] was then released inside the brood chamber, temporarily sedating the fish. Fish were placed in mesh cloth collection bags and brought to the surface.

On shore, we sexed the fish and measured standard length (SL) to the nearest millimeter and body mass to the nearest 0.001 g. In total, we collected 41 dominant breeding males and 62 subordinate helper males from the 55 groups we studied, and every group had a breeding pair and at least one helper. In some cases, the whole group was collected, whereas in other cases only the breeding pair or only the helpers were collected from a group. Fish were anesthetized in benzocaine, quickly sacrificed via cervical severance, and their testes were removed.

Sperm analysis

Testes were placed on a dry glass microscope slide and weighed, taking care to avoid contact with water or mucus. Relative gonad investment was measured using the gonadosomatic index (GSI = [gonad mass/body mass] × 100%). Testes were then split open with a scalpel, allowing access to milt. A drop of milt was placed in a 2-ml microcentrifuge tube and activated by quickly adding 0.25 ml of lake water (previously boiled to exclude microorganisms and allowed to cool to ambient temperature). The video recordings (see below) began as soon as lake water was added to the sample. The water/sperm mixture was agitated for 1–2 s, and a 60-μl subsample was immediately placed in a 1-mm deep well on a slide, with a cover slip covering half of the depression (see Liley et al., 2002 for similar methods). Videos of sperm motility were captured at 60 frames/s using a PixeLINK Megapixel PL-A662 digital video camera (PixeLINK, Ottawa, Ontario, Canada) mounted on a Leica DME light microscope (Leica Microsystems Inc., Buffalo, New York, USA) at 200× magnification. Images were recorded using PixeLINK PL-A600 Series Camera Software (v. 3.1, PixeLINK).

Videos taken in the field were brought back to the lab where sperm velocity was measured using a CEROS (v.12) video sperm analysis system (Hamilton-Thorne Research, Beverly, Maine, USA). For each male, sperm velocity was quantified for 1 s at 2, 5, and 7 min postactivation. We analyzed only those spermatozoa whose forward movement was recorded for at least 0.33 s ($\geq$20 frames; see Burness et al., 2004, and Lahnsteiner et al., 1998, for a similar criterion). The median sperm velocity (median smooth path velocity [VAP]) for all spermatozoa recorded at each time period after activation (mean ± SE: 40 ± 4 cells per time period; range: 6–279).

The duration of sperm movement was measured as the time since activation at which 95% of the sperm no longer exhibited forward movement (see Gage et al., 1998, 2002; Hoysak and Liley, 2001). Two, five, and seven minutes after sperm activation, sperm motility was evaluated by eye on a 0–6 scale, similar to that described by DeFraipont and Sorensen (1993) and Hoysak and Liley (2001): 0 = no motility; 1 = 1–10% of sperm showing forward movement (very low); 2 = 11–29% (low); 3 = 30–49% (moderately low); 4 = 50–74% (moderately high); 5 = 75–94% (high); and 6 = 95–100% (very high). Two independent observers scored both the duration and rank of sperm motility (2, 5, and 7 min postactivation), and mean estimates were used in further analyses. Repeatability between observers, using the intraclass correlation coefficient (r_I), was high (in all cases $r_I \geq .80$, $p < .0001$).

Statistical analysis

Statistical analyses were performed with JMP (version 5.1, SAS Institute Inc., 2004) and Resampling Stats for Excel (version 3, Resampling Stats Inc., 2004). Nonparametric statistics were used when the data were not normally distributed and could not be transformed to correct this; all rank data were corrected for ties. To distinguish between the effects of helper status and body size on gonadal investment, we performed multiple regression analyses using body length (log-transformed SL) as a measure of body size and coded helper status as an ordinal variable (1–6) in the model. We reached the same conclusions using soma mass (body mass−testes mass) as a measure of body size. To avoid pseudoreplication, all statistical analyses of sperm characteristics were performed using a single (median) value from each male. All other measures are presented as mean ± SE. To compare the slopes of reduced major axis (RMA) regressions, we used a randomization test (Manly, 1991) with 1000 iterations to generate 95% confidence limits (CL) for each slope, as well as a sampling distribution of differences between slopes, which was compared to the expected difference of zero that would be obtained if there was no difference between the slopes.

RESULTS

Gonadal investment: testis size and GSI

Breeder males ($n = 41$) had both larger testes mass (Figure 1a) and higher GSI (Figure 1b) than helper males ($n = 62$) (t tests: testes mass, $t = 13.9$, $p < .0001$; GSI, $t = 2.8$, $p = .006$). The average breeder testes mass was 3.6 times that of helpers at the top of the dominance hierarchy (H1; Figure 1a). Testes mass did not differ among helpers in any position in the dominance hierarchy (ANOVA, $F_{5,56} = 1.64$, $p = .17$; Figure 1a), though the testes of male helpers at the top of the hierarchy (H1) were about twice the mass of the other helpers' testes.

Helpers at the bottom of the dominance hierarchy had significantly larger GSI than those at the top of the hierarchy (log GSI data, $F_{5,56} = 2.65$, $p = .03$; Figure 1b), but this effect disappeared when the effects of body size were controlled statistically (analysis of covariance, $F_{5,55} = 1.52$, $p = .20$), suggesting that size and not helper dominance status determined GSI. Thus, within helpers, GSI was negatively correlated with body length (SL) ($r = -.42$, $n = 62$, $p = .001$; Figure 2a). Within breeders, however, GSI and body length (SL) were not correlated ($r = .13$, $n = 41$, $p = .42$; Figure 2a).

The relation between testes mass and soma mass (both log transformed) differed significantly between breeders and helpers (randomization test comparing RMA slopes, $p < .001$). In breeders, the slope (v) of the RMA regression ($v = 2.21$, 95% CL = 1.72–2.93, $n = 41$) was significantly >1.0, whereas the slope of the RMA regression in helpers was not ($v = 1.04$, 95% CL = 0.85–1.29, $n = 62$; Figure 2b). Thus, testes mass was larger relative to body size in breeders than in helpers.

Male breeders invested more in testes (i.e., had higher GSI values) when they were in larger groups ($r = .42$, $n = 34$, $p = .02$; Figure 3a). This was not simply a result of breeders being larger in larger groups as breeder size and group size were not correlated in our sample (body mass, $r = .20$, $n = 34$, $p = .25$; SL, $r = .30$, $n = 34$, $p = .09$). Further, controlling for body mass had little effect on the relation between group size and breeder GSI (partial $r = .44$, $n = 34$, $p = .03$). Breeder GSI was also significantly correlated with the number of male ($r = .35$, $n = 32$, $p = .05$; Figure 3b) but not female ($r = .32$, $n = 32$, $p = .08$) helpers in the group.

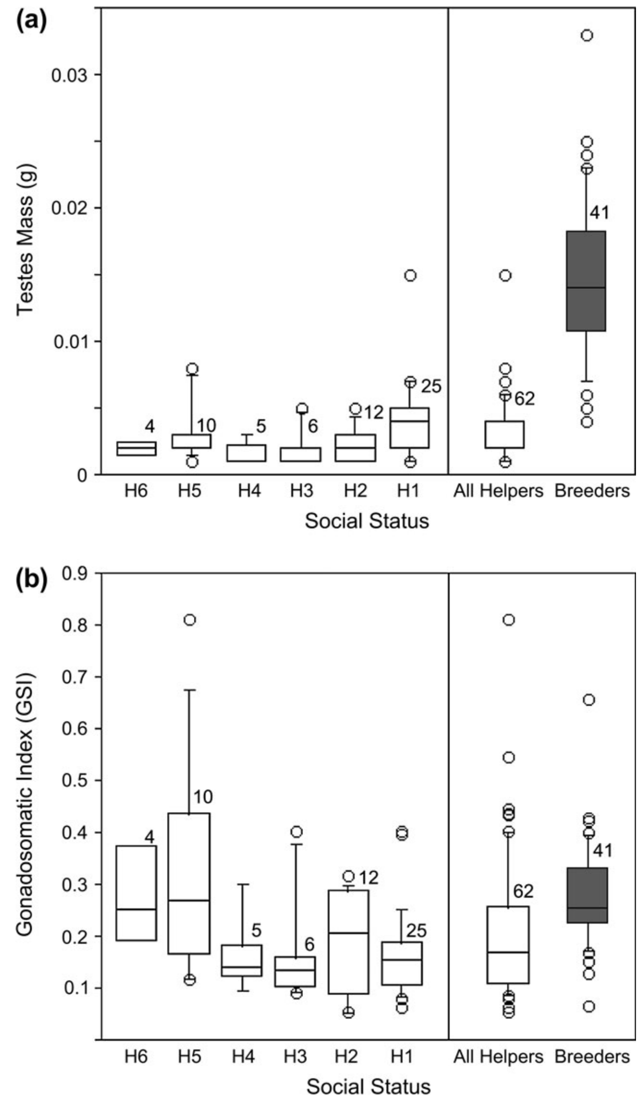

Figure 1
(a) Testes mass and (b) gonadal investment (GSI) in breeder (shaded boxes) and all helper (open boxes) males combined, and across the helper dominance hierarchy (H1–H6). Box plots show 10th, 25th, 50th (median), 75th, and 90th percentiles as horizontal lines plus all data points outside this range. Sample sizes are indicated above each box.

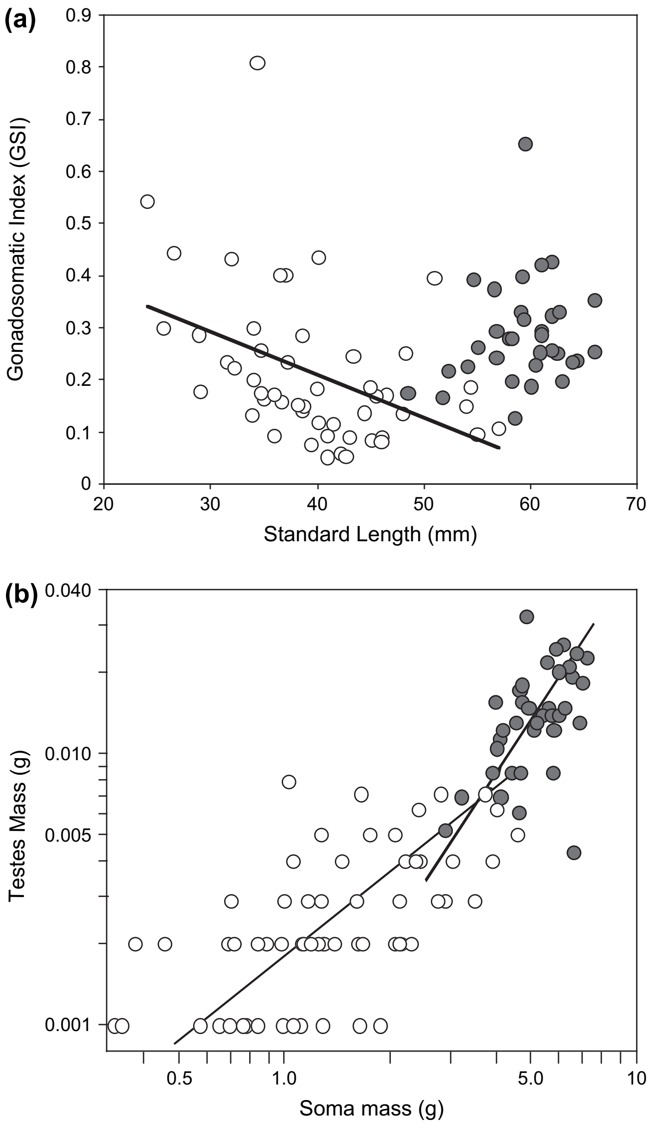

Figure 2
(a) Gonad investment (GSI) of breeder (●, $n = 41$) and helper (○, $n = 62$) males in relation to body size. (b) Breeder ($y = 0.02x - 0.01$) and helper ($y = 0.003x - 0.0002$) testes mass in relation to body mass (both log-transformed). Model II (RMA) regressions are shown.

Sperm swimming speed

In both breeders and helpers, sperm velocity (VAP) declined significantly at the three time periods measured after activation (Breeders: Kruskal-Wallis sign rank test, $H = 36.25$, $n = 41$, $p < .001$; Helpers: $H = 42.6$, $n = 62$, $p < .001$; Figure 4a). In both breeders and helpers, mean VAP at 5 min after activation was less than 75% of the VAP at 2 min.

The mean sperm velocity (VAP) of breeders was higher than that of helper males (Figure 4a) by between 2 and 6 μm/s at all times measured after activation. This difference between breeder and helper sperm velocities was not significant at 2 min postactivation ($U = 1.82$, $n_b = 32$; $n_h = 48$, $p = .18$) but was significant at both 5 ($U = 5.21$, $n_b = 32$; $n_h = 44$, $p = .02$) and 7 min ($U = 4.77$, $n_b = 27$; $n_h = 33$, $p = .03$) after sperm activation (Figure 4a).

Sperm velocity at each time postactivation was not correlated with breeder size (e.g., Figure 4b). In helpers (controlling for status), sperm swimming speed did not vary with body size (SL) at any of the times that speed was measured (multiple regressions: 2 min, $F_{1,41} = 1.15$, $p = .37$; 5 min, $F_{1,39} = 0.67$, $p = .67$; 7 min, $F_{1,28} = 0.69$, $p = .66$).

Sperm longevity

The mean duration of sperm motility for all fish (breeders and helpers) was 7.03 ± 0.23 min ($n = 75$, range 124–766 s). Breeder sperm swam significantly longer than sperm from helpers ($t = 3.14$, $n_b = 32$; $n_h = 46$, $p = .003$; Figure 5a). Within breeders, there was no relation between body length (SL) and sperm longevity ($r = .05$, $n = 32$, $p = .78$), but within helpers, sperm swimming duration was positively correlated with SL ($r = .31$, $n = 46$, $p = .04$; Figure 5b). Similarly, sperm longevity increased up the dominance hierarchy ($F_{5,45} = 2.84$, $n = 43$, $p = .03$), with sperm from H1 males swimming significantly longer than sperm from H6 (Tukey-Kramer test, $p < .05$; Figure 5a). Controlling for social status, helper sperm longevity was

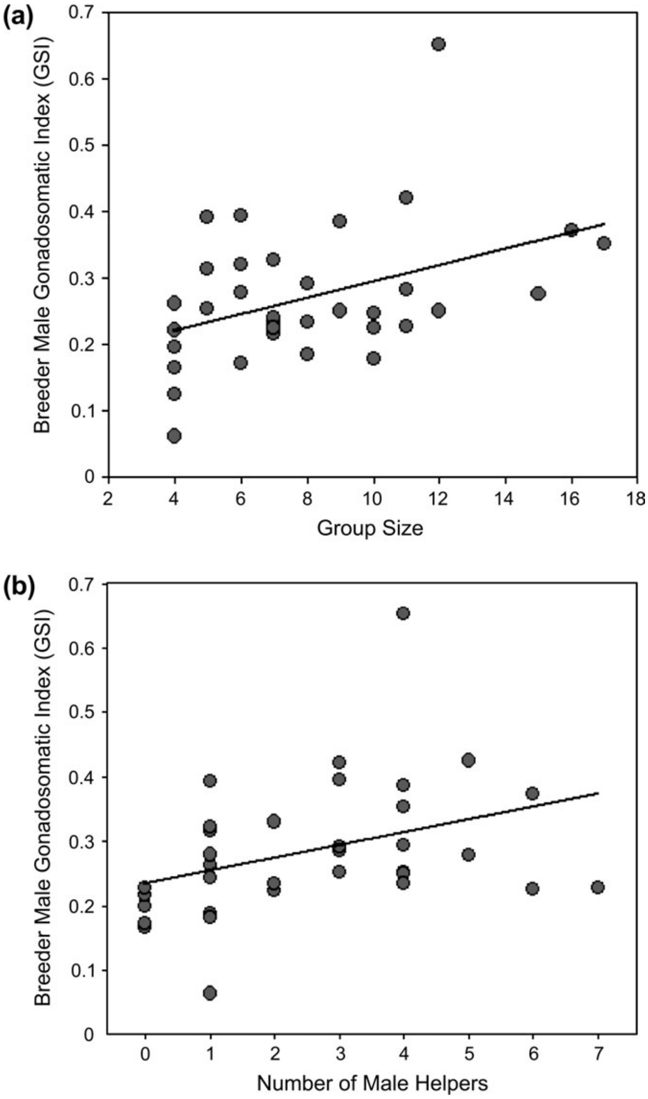

Figure 3
Breeder male GSI (n = 34) increased with (a) total group size ($y = 0.01x + 0.17$) and (b) the number of male helpers present in the group ($y = 0.02x + 0.23$).

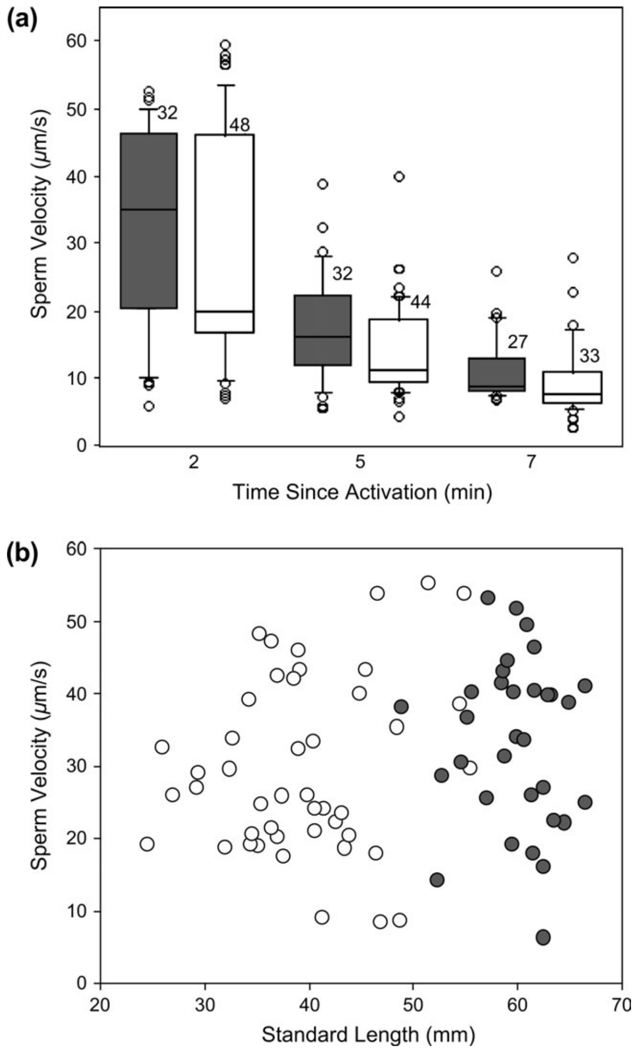

Figure 4
(a) Breeder (shaded boxes) and helper (open boxes) sperm swimming velocities (VAP) decreased after activation (helpers of all social ranks pooled). Box plots as in Figure 1. (b) Median sperm swimming velocity in breeder (●, n = 32) and helper (○, n = 42) males in relation to body length (SL) at 2 min postactivation (helpers of all social ranks pooled; $r_s = -0.08$, n = 41, p = .66). Similar results were found when examining sperm velocities at 5 ($r_s = -0.23$, n = 32, p = .20) and 7 min postactivation ($r_s = -0.29$, n = 27, p = .14).

not related to body size (multiple regression, standardized beta = 0.05, $F_{1,36} = 0.08$, p = .78) but was positively correlated with testes mass (standardized beta = 0.31, $F_{1,36} = 4.71$, p = .04).

Percentage of motile sperm

The proportion of sperm exhibiting forward movement decreased significantly over time since activation in both breeders (H = 41.1, n = 33, $p < .0001$) and helpers (H = 67.8, n = 48, $p < .0001$; Figure 6). Moreover, at each time period analyzed, a significantly greater proportion of breeder sperm was motile compared to helper sperm (2 min after activation: U = 5.35, n_b = 33; n_h = 48, $p < .02$; 5 min: U = 7.63, n_b = 33; n_h = 44, $p < .005$; 7 min: U = 11.44, n_b = 27; n_h = 35, $p < .0007$; Figure 6).

Group size and aggression

All aggression was directed down the dominance hierarchy by breeders toward helpers, or from more dominant helpers (e.g., helper 1s) toward more subordinate helpers (e.g., helper 2s). While breeders (1.84 ± 0.45 acts/10 min) and helpers (2.19 ± 0.52) performed aggression toward members of their own social groups at roughly equal frequencies (U = 0.14, p = .71), helpers performed submissive behavior at a much higher rate (2.70 ± 0.67) than breeders (0.44 ± 0.13; U = 12.0, p = .0005).

None of the sperm characteristics measured were correlated with group size or helper sex ratio within each group ($p > .05$ in all cases).

DISCUSSION

Our investigation of gonadal investment patterns in male breeders and helpers provides support for the idea that reproductive suppression occurs in *N. pulcher.* First, helpers had smaller absolute testes mass (Figure 1a) and less gonadal investment (Figure 1b) than breeders, suggesting that they would have less sperm available per ejaculate (Gage et al.,

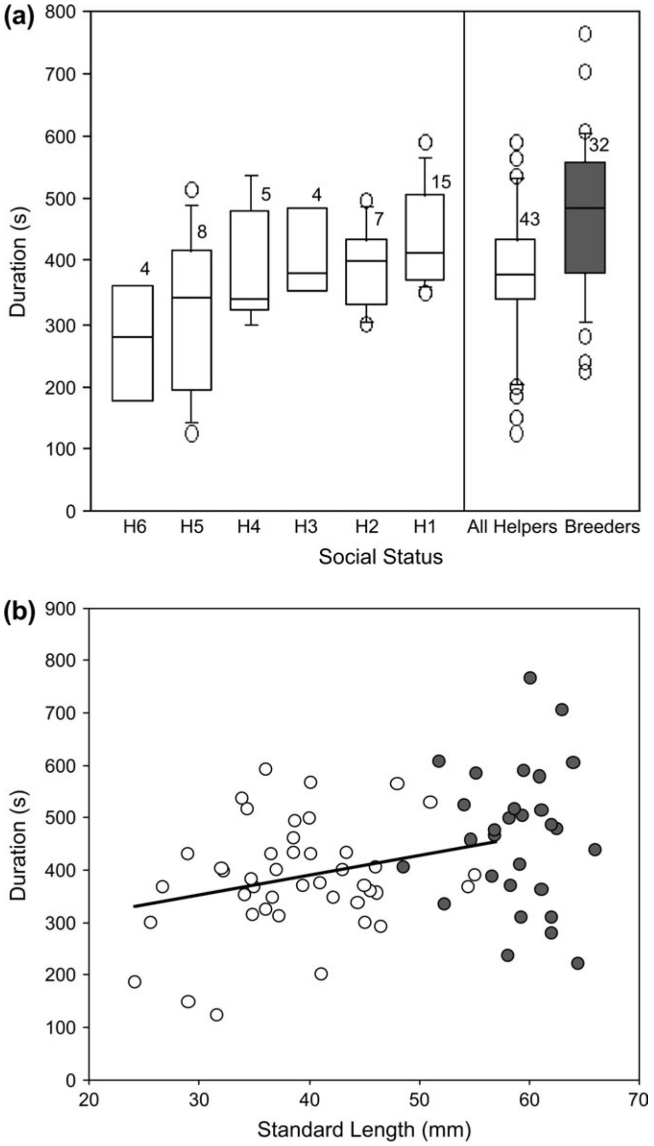

Figure 5
(a) Duration of sperm motility in all breeder (shaded boxes) and helper (open boxes) males and across the dominance hierarchy (H1–H6). Box plots as in Figure 1. (b) Sperm swimming duration of breeder (●, $n = 32$) and helper (○, $n = 46$, $y = 41.9x + 218.9$) males in relation to body length (SL).

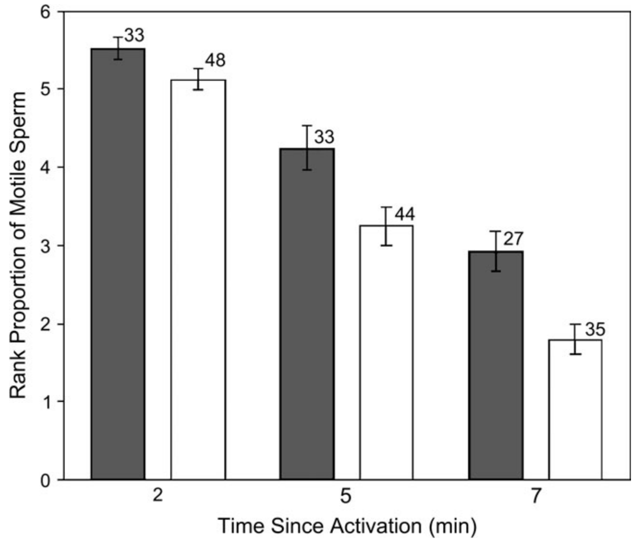

Figure 6
The percentage of sperm that was motile 2, 5, and 7 min after activation in breeder (shaded boxes) and helper (open boxes) males. Sample sizes (above boxes) vary across time periods because the sperm of some individuals were no longer swimming at 5 min and 7 min postactivation, and these males were not included in the analyses at those times.

1995). Second, helpers had slower swimming sperm (Figure 4), shorter lived sperm after activation (Figure 5), and a lower percentage of motile sperm (Figure 6) than breeders, suggesting that their sperm would be less competitive (Hoysak and Liley, 2001). Finally, breeding males were aggressive toward helpers, and helpers were much more submissive than breeders, suggesting that breeder males may behaviorally control reproduction in the helpers.

Among helpers, testes mass increased isometrically with body size (Figure 2b), whereas among breeders this relation was allometric, suggesting that testes mass increases at a faster rate than the soma in breeders (Figure 2b). As a result, the relative investment in gonads (GSI) decreased as helper size increased, but gonadal investment increased proportionately to body mass in breeders (Figure 2a). These results suggest that helpers put energy into somatic growth, whereas breeders divert energy into increasing gonadal development. All these results point to helpers, in general, being reproductively inferior to breeders, possibly as a result of active suppression via breeder aggression.

There was also some evidence that, as helpers get larger, their sperm characteristics more closely approximate those of breeders. For example, sperm longevity (Figure 5) increased with body size. On the other hand, the testes mass of even the largest helpers was small relative to that of breeders (Figure 1a,b). As sperm density and the density of sperm around the egg increases fertilization success both in vitro (Hoysak and Liley, 2001, Liley et al., 2002) and in vivo (Marconato and Shapiro, 1996; Marconato et al., 1995) and predicts success in sperm competition trials (Parker et al., 1990), the reduced gonadal investment observed in helper males likely limits any potential reproductive output. Breeding males devoted more to gonadal investment in larger groups (Figure 3a), possibly in response to increased sperm competition, as, in large groups (with more male helpers), the threat of sperm competition (i.e., sneak fertilizations) for breeders might be expected to select for breeders to increase gonadal investment. However, sperm competition theory suggests that selective pressure from sperm competition should be greatest on those performing sneak fertilizations (putatively the helper males). Thus, we would expect to see a pattern strikingly different to the one observed if there was intense sperm competition within groups, namely, a higher quality ejaculate in males in the disfavored role (Møller, 1988, 1989; Parker, 1990a), but there was no evidence for this.

The smaller, but reproductively mature, helpers that are at the bottom of the dominance hierarchy could possibly sneak fertilizations. Within teleost species exhibiting parasitic fertilizations, cuckolder males are typically female mimics (satellites), or are physically small and inconspicuous sneakers, to avoid retaliation from dominant individuals (Taborsky, 1994). Small *N. pulcher* helpers with relatively large gonadal investments would certainly fall into the latter category. However, based on their small absolute testes mass and inferior gametic traits, it seems unlikely that small helpers would perform sneak fertilizations. Furthermore, the higher GSI values observed in smaller helpers (Figure 1b) is driven by the fact that testes mass does not change significantly as helper size increases. As a result, testes mass remains constant across the dominance hierarchy (Figure 1a). In addition, small helpers may need to avoid inbreeding to a greater extent compared to larger helpers because smaller helpers are more closely related to breeders than large helpers because of frequent breeder turnover (Stiver et al., 2004; Taborsky and Limberger, 1981). Also, the costs of group expulsion may be higher for smaller individuals, who may be more vulnerable to predators and other ecological pressures, such as food shortage (Paine, 1976). To determine whether these smaller helpers ever father any young, a more thorough paternity analysis on a large number of groups from the field is required.

As mentioned in the Introduction, only a few studies have so far examined sperm characteristics of males utilizing different reproductive tactics in fish. Males engaging in sneak fertilizations in both the Atlantic salmon and bluegill have different sperm characteristics than dominant males (Burness et al., 2004; Gage et al., 1995; Leach and Montgomerie, 2000; Vladic and Järvi, 2001; Vladic et al., 2002; but see Liley et al., 2002). Sneaker males attained high levels of reproductive success in both species, fertilizing 5–40% of eggs in salmon (Hutchings and Myers, 1988; Jordan and Youngson, 1992; Thomaz et al., 1997) and up to 92% of eggs in bluegill (Fu et al., 2001). Therefore, unlike species where sneak fertilizations are common, in *N. pulcher* none of the sperm characteristics of males in the disadvantaged role (helpers) surpassed those of breeders—in fact, we found just the opposite. Therefore, if *N. pulcher* helpers engage in sneak fertilizations, as has been suggested (Dierkes et al., 1999), sperm characteristics have not been influenced. Previous theoretical and empirical research suggests that competition will increase ejaculate quality of the potential sneaks (Burness et al., 2004; Gage et al., 1995; Vladic and Järvi, 2001), but we found the opposite pattern in that helpers had sperm that was less motile and slower than that of breeders. Thus, it seems highly unlikely that helpers were responsible for the mixed paternity (Dierkes P, Taborsky M, and Achmann R, personal communication) observed in wild populations.

The disparity in testes size and GSI between large helpers and breeders may represent an adaptive trade-off between immediate and future reproductive success (Williams, 1966). Helpers may put energy into growth or energy stores, rather than gonad development, subsequently using energy stores to overcome the observed large difference in gonad, and presumably ejaculate, size. Several teleost species exhibit socially mediated gonadal growth (Berglund, 1991; Schultz et al., 1991) and many, including *N. pulcher* (Heg et al., 2004b), are known to exhibit socially modulated growth rates (Borowsky, 1973; Buston, 2003; Hofmann et al., 1999). So, helpers may delay final sexual maturation until a favorable situation is presented (Wickings and Dixson, 1992), at which time rapid sexual maturation may be possible, where gonads grow at a faster rate than the soma (Figure 3b). Indeed, dramatic sexual maturation and gonadal development is observed in many teleosts, over a period of days to weeks, after their acquisition of socially dominant positions in their social group (Hofmann et al., 1999; Munday et al., 1998; Warner and Swearer, 1991).

In contrast to the sperm characteristics reported in other cooperatively breeding species (naked mole-rat, Faulkes and Abbott [1991], Faulkes et al. [1994], Jarvis [1991]; and Damaraland mole-rat, Maswanganye et al., 1999), large subordinate male *N. pulcher* did not have impaired sperm physiology, though they did have much smaller testes size than breeders (Figure 1). Large subordinate males had physiologically equivalent sperm characteristics to breeders, but their relatively small gonads suggest that they are limited in reproductive capacity. Rather than investing in gonadal development, subordinate helpers may invest in strategic somatic growth (Heg et al., 2004b), using stored energy only to rapidly enhance gonad development as breeding opportunities arise. Future work could provide a critical test of this idea by examination of subordinate physiology before and after the assumption of breeder status, focusing in particular on gonad growth and use of stored energy reserves. We found little evidence from male physiology to support the idea that sneak fertilizations by helpers are responsible for mixed paternity in wild *N. pulcher* populations (Dierkes P, Taborsky M, and Achmann R, personal communication). Instead, this mixed paternity may be the result of extragroup fertilizations by neighboring males, as has been seen in other cooperatively breeding species (Cant et al., 2002; Double and Cockburn, 2000; Hatchwell et al., 2002).

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